



## **Metagenomic analysis of microbial communities in rapid sand filter treating groundwater. Community diversity and metabolic potential**

**Palomo, Alejandro; Rasmussen, S.; Sicheritz-Pontén, Thomas; Smets, Barth F.**

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## **Metal specific modulation of community permissiveness towards broad host range plasmids through stress**

**Klümper, Uli; Brandt, K.K.; Dechesne, Arnaud; Riber, Leise; Sørensen, S.; Smets, Barth F.**

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**FEMS-0807**  
**Giant viruses**

**Giant viruses**

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More than a century ago, the notion of "virus" was introduced to designate infectious agents invisible to the light microscope and capable of passing through "sterilizing" filters. In addition to their extremely small size, most viruses studied over the years also exhibited minimal genomes and gene contents, almost entirely relying on cell-encoded functions to multiply, as expected from absolute intracellular parasites. Unexpectedly, the last ten years have seen the discovery of 4 different families of eukaryotic "giant viruses" exhibiting particles of cellular dimensions as well as gene contents overlapping in size with that of bacteria and some parasitic eukaryotes. Although all presently known giant viruses have been isolated using *Acanthamoeba* as laboratory host, related members are now starting to be found in other protozoa, such as marine picoplankton species the population of which they regulate. Representatives of two families of giant viruses have been revived from a layer of Siberian permafrost radiocarbon dated from 30.000 years ago, raising concern that pathogenic viruses from long past epidemics might also remain infectious and resurface in circumpolar regions as a consequence of global warming and industrial exploitations.

The unexpected abundance, ubiquity and diversity of giant viruses, as well as the alien nature of their gene contents deeply challenge conventional conceptions about the origin and evolution of all DNA viruses and raise the question of their evolutionary relationship with the 3 cellular domains forming today's Tree of Life, and possibly other long extinct cellular lineages.

It will conclude by discussing the nature of the epistemological barrier that led to the delayed discovery of these highly visible viruses, most of which were spotted 10 year before their viral nature was eventually recognized.

**FEMS-1062**  
**(Human) microbiome**

**(human) microbiome**

J.K. Jansson<sup>1</sup>

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The performance of the human microbiome is perturbed in unknown ways by a variety of factors including disease and diet. Here we aimed to determine the impact of inflammatory bowel disease (IBD) and a resistant starch diet on the gut microbiome community composition and functional potential by application of an array of omics technologies: 16S rRNA sequencing, metagenomics, metaproteomics and metabolomics. The first studies focused on a Swedish twin cohort and a longitudinal cohort for IBD. Specific bacterial species, proteins and metabolites correlated with different sub-phenotypes of the IBD; including a reduced amount of normal beneficial microbes as well as proteins involved in butyrate metabolism, suggesting potential biomarkers of IBD. For the dietary study, subjects transitioned from a baseline diet to one with low or high amounts of resistant starch. Specific bacteria, proteins and metabolites were shown to significantly vary in abundance when comparing baseline to the high resistant starch diet. Together these studies exemplify the use of omics to provide potential bioindicators of specific physiological states of importance to human health.



**FEMS-0959**

**Plant-fungal pathogens**

**Plant-fungal pathogens**

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Fungi are notorious plant pathogens that cause serious diseases on natural vegetations and crop plants. The majority of plants, however, is not infected by fungal pathogens as they recognize pathogen-associated molecular patterns (PAMPs) like chitin or glucans by pattern recognition receptors that mediate PAMP-triggered immunity (PTI), a basal defense response effective against potential fungal pathogens. Successful fungal plant pathogens secrete effectors to suppress PTI and alter host plant physiology enabling them to infect plants. In turn, plants have evolved immune receptors that recognize effectors resulting in effector-triggered immunity (ETI) including the hypersensitive response, effective against biotrophic fungal plant pathogens that require living cells to feed on. Other fungal pathogens are hemi-biotrophic, which start infection as a biotroph, but after having colonized the host tissue can also feed on death tissue. Necrotrophic fungal pathogens kill host tissue before they start to feed on it. Co-evolution between fungal pathogens and their hosts had led to the development of numerous effectors in fungal plant pathogens and corresponding resistance proteins in host plants, which has generated an arms race genetically described by the gene-for-gene concept. Resistance genes encoding resistance proteins have now been cloned and are successfully transferred to crop plants by classical breeding or as transgenes stapled into one plant cultivar.

**FEMS-0682**

**Resistome**

**Ranking risks in resistomes. The intrinsic resistome**

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Before the antibiotic era, bacterial pathogens were by definition susceptible to the antibiotics that pharmaceutical companies have developed to fight infections. Nevertheless, soon after the introduction of antibiotics for therapy, resistant isolates emerge and spread among the population. Resistance can be acquired either because of mutations or by acquiring resistance genes from a donor strain. Since pathogens were susceptible, resistance genes should come from non pathogens and, most likely from environmental microorganisms. Indeed, the analysis of metagenomes from very different ecosystems has shown that antibiotic resistance genes are ubiquitously found in any place in Earth. These genes (the resistome) can be transferred to human pathogens, then constituting a risk for human health. However, if all ecosystems contain resistance genes, all of them will be risky, which is the same as saying that no one has a relevant differential risk. In the presentation the possibility of ranking risk of resistomes will be discussed. The ranking is based in the likelihood that the finding of a specific gene in a given ecosystem constitutes a real risk for human health. One of the relevant aspects to take into consideration is whether those genes belong to the transferrable resistome (the mobilome) or are just a part to the set of elements that contribute to the characteristic phenotype of susceptibility to antimicrobials of a given microorganisms (its intrinsic resistome). Current information on the elements forming part of the intrinsic resistome will be discussed in the presentation.

Further reading:

<http://www.nature.com/nrmicro/journal/vaop/ncurrent/full/nrmicro3399.html>

**FEMS-2848**

**Resistome**

**Delineating the dissemination of antibiotic resistance genes within microbial communities**

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Antibiotic resistance is a growing public health concern that negatively impacts treatment outcomes of most infections. The issue is projected to grow substantially in the coming decades and new approaches are needed to curb the spread of resistance. Notably, resistance evolves through a complex interplay between residents of most microbial communities and organisms causing disease. In spite of significant efforts using a variety of experimental and computational approaches our understanding of the dissemination of resistance genes is still limited. To institute preventative regulations on key environments harboring microbial communities that influence resistance gene dissemination general and unbiased approaches are necessary. Yet, to date our ability to assess the impact of specific microbial communities on the dissemination of antibiotic resistance is inadequate. Recent improvements in experimental and computational approaches aimed at addressing this overall goal are discussed with a focus on key environments believed to influence resistance gene dissemination.

**FEMS-2894**

**Resistome**

**COMPARATIVE GENOMICS TO INFER THE ANTIBIOTIC RESISTANCE  
MOBILOME IN CLONALLY RELATED AND UNRELATED STRAINS**

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<sup>1</sup>*Medical Microbiology, University Medical Center Utrecht, Utrecht, Netherlands*

The introduction of antibiotics revolutionized human medicine and agricultural industry, but their success has created a public health threat caused by antibiotic-resistance (ABR). Indisputably, human use of antibiotic has contributed to the expansions of ABR. At the same time it is becoming increasingly clear that antibiotic use in animal husbandry has also created a large reservoir of ABR with increasing risk of spill over of ABR from animals to humans. Whole genome sequencing (WGS) has revolutionizing research into transmission of ABR by providing the optimal resolution to infer epidemiological linkage of multidrug resistant isolates and their resistance genes and plasmids. However, frequently used WGS technologies rely on the generation of sequences with short-read length resulting in many contigs of assembled reads without easy assorting contigs to plasmids and the main chromosome. We used a novel algorithm called PLACNET that identifies plasmid contigs in WGS projects to study genetic relatedness of ESBL-genes containing plasmids from animal and human origin. Plasmid reconstructions, validated by long-read DNA sequencing, indicated that genetically unrelated human isolates shared virtually identical plasmids with animal isolates.

Another potential drawback of WGS for studying transmission of ABR is the lack of a standardized and expandable classification scheme since most WGS-based analyses rely on SNP detection from reads mapped to reference genomes that are often distinct for different epidemiological studies. This limitation may be overcome by using a gene-by-gene comparison approach just like classical MLST but with an important extension of the numbers of analysed genes from seven to several hundreds/thousands. We developed an extended, core genome MLST scheme and showed its value in determining the clonal relatedness of multi-resistant *Enterococcus faecium* from hospital outbreaks in three different countries.

**FEMS-2388**

**Resistome**

**Networks of Exchanging Antibiotic Resistance in Human and Environmental Microbiota**

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While the most acute effects of increasing antibiotic resistance in pathogens are observed in clinical settings, it is becoming increasingly clear that the evolution and transmission dynamics of resistance gene dissemination is an ecological problem. Indeed, steady use and abuse of antibiotics over the past century in food animals, humans, and the environment has provided substantial selective pressure for enrichment of resistance genotypes in each of their associated microbiomes. An over-reliance on culture-based methods, the standard in the study of clinical resistance, has vastly underestimated these reservoirs of resistance genes (or 'resistomes'). To address this issue, we have recently developed high-throughput metagenomic functional selections, aided by next-generation sequencing, to characterize resistomes encoded by the microbiota of healthy human adults and children as well as diverse soils. Hundreds of resistance genes we identify from specific taxa in these different microbial communities are identical to resistance genes found in major human pathogens, indicating recent genetic exchange between these microbes. We also find thousands of functionally validated resistance genes which are genetically novel, but flanked by genes involved in horizontal gene transfer, including transposases and integrases. Together, these findings highlight the substantial antibiotic resistome encoded by microbes from diverse environments, which is available for exchange with pathogens, with the potential to severely exacerbate the problems with clinical resistance.

## **FEMS-2417**

### **Pathogenicity and cell surface**

#### **Protein secretion and secreted proteins in *Neisseria meningitidis***

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<sup>1</sup>*Molecular Microbiology, Utrecht University, Utrecht, Netherlands*

*Neisseria meningitidis* is a strictly human pathogen that colonizes the nasopharynx as a commensal but occasionally crosses the epithelial barriers to cause sepsis and/or meningitis. Proteins secreted by this Gram-negative diplococcus are considered to be important for colonization and/or infection. Of six protein secretion systems widely disseminated among Gram-negative bacteria, only two are present in *N. meningitidis*, i.e. type I and type V, the latter being subdivided in autotransporters and two-partner secretion (TPS) systems.

We are investigating the functions of type V secreted proteins and found that many of them are involved in interbacterial interactions. Neisserial IgA protease was the first autotransporter ever described. Besides its protease domain, also a ~40-kDa polypeptide of unknown function, the  $\alpha$ -peptide, is transported to the bacterial cell surface. Our results demonstrate that this positively charged  $\alpha$ -peptide and a cell-surface-exposed lipoprotein, the neisserial heparin-binding antigen (NHBA), are involved in biofilm formation by binding extracellular DNA, an important matrix component of meningococcal biofilms. Another autotransporter, the protease NalP, inhibits biofilm formation by cleaving the  $\alpha$ -peptide of IgA protease and NHBA from the cell surface. Yet another autotransporter, designated AutA, is only expressed in a limited number of meningococcal isolates. Its expression causes bacterial autoaggregation and influences the architecture of the biofilms formed.

The substrates of the meningococcal TPS systems are large proteins. These proteins were found to contain a small toxic domain at their C-terminus that inhibits the growth of related bacteria. Similar toxic domains were found at the C-terminus of another family of proteins, MafB. MafB proteins are transported to the cell surface via a protein, MafA, representing a novel protein secretion system. Like the TPS system, the MAF system is involved in interbacterial competition.

**FEMS-1153**

**Pathogenicity and cell surface**

**How a surface-exposed lipoprotein functions as an envelope stress sensor in enterobacteria**

*S.H. Cho<sup>1</sup>, J. Szewczyk<sup>1</sup>, C. Pesavento<sup>2</sup>, A. Typas<sup>2</sup>, J.F. Collet<sup>1</sup>*

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### **Background**

The cell envelope protects bacteria from their surroundings. Breaches in its integrity or defects in its assembly are sensed by signal transduction systems, allowing cells to rapidly adjust. The Rcs phosphorelay responds to outer membrane (OM)- and peptidoglycan-related stress in enterobacteria. An OM lipoprotein, RcsF, is required for sensing and transducing the stress signals to downstream components in yet unidentified manners.

### **Objectives**

We set out to discover how RcsF detects stress and activates the Rcs signalling cascade.

### **Methods**

We used a combination of methods, including site-specific photo-crosslinking, co-immunoprecipitation and fluorescence microscopy.

### **Conclusions**

We found that RcsF interacts with BamA, the major component of the OM beta-barrel assembly machinery. BamA funnels RcsF to the beta-barrel OmpA, allowing RcsF to reach the cell surface in an unresponsive-to-stress conformation. This spatially separates RcsF from the inner membrane-located IgaA, which we show is the next component of the cascade. Stress conditions prevent RcsF from interacting with BamA, allowing it to reach IgaA. Thereby the BamA-RcsF interaction acts as a checkpoint for lipoprotein transport to the cell surface, which we propose is mediated by BamA itself.

## **FEMS-2857**

### **Pathogenicity and cell surface**

#### **Adhesion, invasion, evasion. The many functions of surface proteins of *Staphylococcus aureus***

T. Foster<sup>1</sup>

<sup>1</sup>*Microbiology, Trinity College Dublin, Dublin, Ireland*

*Staphylococcus aureus* colonizes the moist squamous epithelium of the nasal cavity of about 20% of the population where it resides as a harmless commensal. It can cause infections ranging from superficial skin and soft tissue infections to those that are invasive and seriously life threatening. The surface of *S. aureus* is decorated with up to 24 cell wall anchored (CWA) proteins that are covalently linked to peptidoglycan by sortase. CWA proteins perform a variety of functions *viz* adhesion to host cells and tissue, evasion of innate and adaptive immune responses, invasion of host cells, acquisition of iron from haemoglobin, biofilm formation and stimulating inflammation. They can be classified into six groups based on structural and functional characteristics. The largest class are called microbial surface components recognising adhesive matrix molecules (MSCRAMMS) which have at their N terminus an A domain containing two adjacent subdomains each comprising IgG-like folds. MSCRAMMS bind to short peptide sequences within host proteins by the dock latch lock mechanism. Clumping factor A is the archetypal MSCRAMM which binds to the C-terminus of the gamma-chain of fibrinogen. The N-terminal A domains of MSCRAMMS are displayed on the cell surface aided by unfolded flexible stalks. In the case of ClfA and many other MSCRAMMS the stalk comprises serine-aspartate dipeptide repeats. In fibronectin binding proteins the linker for the A domain comprises fibronectin binding repeats. Several CWA proteins are being tested as components of vaccines aimed at reducing *S. aureus* infections



**FEMS-3163**

**Pathogenicity and cell surface**

**Role of the ESX-5 protein secretion system in functioning of the mycobacterial cell envelope and in virulence**

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Mycobacteria use type VII secretion (T7S) systems to secrete proteins across their complex cell envelope. Pathogenic mycobacteria, such as the notorious pathogen *Mycobacterium tuberculosis*, have up to five of these secretion systems, named ESX-1 to ESX-5. At least three of these secretion systems (ESX-1, ESX-3 and ESX-5) are essential for mycobacterial virulence and/or viability. Elucidating T7S is therefore essential to understand the success of *M. tuberculosis* and other pathogenic mycobacteria as pathogens, and could be instrumental to identify novel targets for drug- and vaccine-development. Recently, significant progress has been achieved in the identification of T7S substrates. We have identified a general motif (YxxxD/E) that is required for secretion. Surprisingly, this motif does not determine system specificity. Secretion of substrates is also dependent on the function of dedicated chaperones. We are currently determining whether these chaperones are involved in system specificity. In addition, a start has been made with unraveling the mechanism of secretion and the structural analysis of the different subunits. We have identified the membrane translocation complex of both the ESX-1 and ESX-5 system. Finally, one of the T7S systems, ESX-5, is only present in slow-growing mycobacterial species and is responsible for the secretion of dozens of substrates. We have recently shown that ESX-5 substrates play a role both in virulence and in nutrient uptake. The latter characteristics could be linked to its exclusive presence in slow-growing mycobacteria.

**FEMS-2868**

**Yeast biotechnology Offered by FEMS Yeast Research**

**Engineering central metabolism in yeast for production of fuels and chemicals**

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<sup>1</sup>*Biotechnology, Delft University of Technology, Delft, Netherlands*

*Saccharomyces cerevisiae* is a robust industrial micro-organism and a popular platform for metabolic engineering. Breath-taking advances in synthetic biology have greatly increased the pace and ease with which biochemical pathways towards non-native compounds can be expressed in this yeast. For environmentally and economically sustainable production of such compounds, it is essential that product yields on (sugar) substrate are as close to their thermodynamic maxima as possible. This not only requires 'tuning' of the non-native product pathways, but also optimization of their interaction with central metabolism, for example for the generation of product precursors and for redox balancing. While central metabolism of *S. cerevisiae* and its regulation have been shaped in aeons of natural evolution to improve competitiveness in natural environments, several key aspects of its redox and energy metabolism are clearly suboptimal for industrial application. In this presentation, I will discuss novel metabolic engineering strategies to address these limitations and, thereby, enable improved yields of existing and novel products of engineered *S. cerevisiae* strains.

## FEMS-0645

### Yeast biotechnology Offered by FEMS Yeast Research

#### Coordination of yeast metabolism through phosphorylation

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While our knowledge on the topology of regulation network steadily increases, we understand much less about the functionality of individual regulation events and their quantitative relevance for controlling a given biological function and eventually a phenotype. Focussing on metabolism, we ask which mechanisms *Saccharomyces cerevisiae* employs to make decisions on the use of different pathways. Although obviously important, transcriptional regulation does not appear to be the major mechanism to control the activity of metabolic pathways in bacteria and yeasts (1). By combining quantitative proteomics, P-proteomics and flux measurements, we instead demonstrated in a pilot study that enzyme phosphorylation, in contrast to transcriptional regulation, frequently contributes to the in vivo control of pathway fluxes in bakers yeast (2). To more broadly investigate the role of phosphorylation in controlling enzyme activity, and thus pathway fluxes, we performed large-scale metabolomics on 118 kinase and phosphate deletion mutants. Combining the metabolome with P-proteomic data, we predicted functional phosphorylation for about 50 metabolic enzymes (3). Different from bacteria, the yeast kinase network is rather interconnected with often multiple kinases acting on a given enzyme, a redundancy that in many cases might obscure functionality assessment. Here I will discuss how we tackle the problem of functionality by two approaches: i) integration of dynamic metabolomics and P-proteomics data, focussing on TOR signalling, and ii) large-scale point mutations in P-enzymes that either mimic permanent phosphorylation (serine to glutamate) or prevent phosphorylation at a given site entirely (serine to alanine).

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## **FEMS-1220**

### **Yeast biotechnology Offered by FEMS Yeast Research**

#### **Quantitative genetics of industrial and new yeast hybrids**

E.J. Louis<sup>1</sup>

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Many yeasts used in industrial fermentations are not pure species but hybrids between 2 or more species. Their advantage is that they can combine useful properties from each of the parental species. The disadvantage for strain improvement is that these hybrids are sterile and therefore not amenable to genetic analysis. Complex trait analysis in these is virtually impossible due to this sterility. The most famous yeast hybrid is the lager yeast, *S. carlsbergensis*, also known as *S. pastorianus*, which actually arose from at least two independent hybridization events between *S. cerevisiae* and another *Saccharomyces* species. Genetic studies and strain improvement has generally been limited to single gene mutations and analysis of rare spore progeny. The recent discovery of the second parental species, *S. eubayanus*, has provided the opportunity to create new hybrids using diverse parental strains in order to enhance genetic analysis. These new hybrids still suffer from the problem of sterility however. There are other hybrids used in industry such as the *S. cerevisiae* by *S. kudriavzevii* hybrids used in wine fermentation. These are less well characterized than the lager strains but share many of the features, such as colder fermentation temperatures, and issues, such as sterility and genome stability. There are also many other hybrids isolated from nature that may or may not have industrial uses. We have recently utilized a tetraploid intermediate strategy to overcome the sterility of hybrids, which allows us to both genetically study existing hybrids as well as create new hybrids such that quantitative genetic analysis can be done. We have successfully restored fertility in a wine hybrid and are exploring the industrial potential of new hybrids, incorporating all the extant species.

## **FEMS-2790**

### **Yeast biotechnology Offered by FEMS Yeast Research**

#### **Lignocellulosic bioethanol – from laboratory proof-of-concept to full scale plant**

L. Raamsdonk<sup>1</sup>, *H. de Bruijn*<sup>1</sup>, *P. Klaassen*<sup>1</sup>

<sup>1</sup>*Biotechnology Center, DSM, Delft, Netherlands*

Lignocellulosic feedstocks are considered to be of great economic and environmental significance for sustainable production of valuable fermentation products. For cost-effective and efficient industrial processes, complete and fast fermentation of all biomass sugars is a prerequisite. In this respect, the main challenge emerging from the use of lignocellulosic feedstocks for ethanol production by *Saccharomyces cerevisiae* is the efficient fermentation of the pentose sugars xylose and arabinose, as these sugars cannot be utilized by natural *S. cerevisiae* strains. Another significant challenge is the inevitable presence of inhibitors, such as furfural and acetic acid, which are formed during pretreatment and hydrolysis of the feedstocks and severely inhibit yeast growth and product formation.

DSM has developed advanced yeast strains by introducing heterologous pathways into robust *S. cerevisiae* hosts which resulted in the ability to ferment xylose and arabinose in lignocellulosic hydrolysates. Subsequently, by the application of evolutionary engineering the total time required to ferment hexoses and pentoses in lignocellulosic sugar mixtures was significantly reduced.

In order to take these developments to the next level, a toolbox for the new generation of advanced yeast strains is in development, in which the main challenges are further addressed by accelerating pentose fermentation through engineered pentose transport, as well as converting lignocellulosic inhibitors and biorefinery waste streams into additional ethanol. A roadmap to implementation of commercial strains in a full scale plant will be presented.

**FEMS-2360**

**Plant microbe interactions Offered by FEMS Microbiology Ecology**

**The phyllosphere microbiota: responses to and impacts on plants**

J.A. Vorholt<sup>1</sup>

<sup>1</sup>*Institute of Microbiology, ETH Zurich, Zurich, Switzerland*

The aerial parts of the plants, which are dominated by leaves, represent one of the largest terrestrial habitats for microorganisms. This habitat, called the phyllosphere, is occupied by a diverse community of microorganisms, which is important for plant health and growth. Most of the phyllosphere inhabitants are not well investigated; however, there is a growing interest to study commensal bacteria to elucidate their interactions with the plants, among each other and to learn how they withstand the hostile conditions of their habitat. A predominance of Proteobacteria, Actinobacteria and Bacteroidetes living in the phyllosphere of numerous plants has been revealed, while metagenomics and metaproteomics approaches gave insights into the general bacterial adaptation strategies to the phyllosphere. Complementary to these cultivation-independent approaches we established a comprehensive strain collection which covers a broad diversity of strains colonizing the model plant *Arabidopsis thaliana*. Targeted studies with model strains allowed us to identify metabolic traits important for plant colonization and to uncover a novel bacterial regulatory system essential for plant colonization which is responsible for the general stress response in Alphaproteobacteria. The establishment of a gnotobiotic system led to the identification of plant probiotic effects of commensal bacteria and candidate genes for plant protection against bacterial pathogens. Moreover, the experimental system paired with synthetic bacterial communities helped identifying plant genes involved in shaping the bacterial community structure.

## **FEMS-2598**

### **Plant microbe interactions Offered by FEMS Microbiology Ecology**

#### **Plant-microbiome interactions in heavy metal polluted environments**

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Metalliferous plants including (hyper)accumulators with tissues containing extremely high trace elements (TE) concentrations are associated with complex microbiomes, which are likely to be important for plant growth under such adverse conditions. Our studies addressed the ecology and microbial community behavior under different conditions as well as the role of plant-associated bacteria for the TE mobilization / availability and plant uptake.

We analyzed microbiomes associated with Zn-accumulating plant *Salix* species, which are applied for phytoextraction applications. Plants were associated with complex rhizosphere microbiomes, which correlated with the plant genotype and which were particularly rich in *Actinobacteria*. Bacteria isolated from Zn accumulating *S. caprea* showed highly different effects on TE mobilization. Several strains decreased bioavailability, whereas others, particularly *Actinobacteria* such as *Microbacterium* spp. strains, increased bioavailability. The mechanism of TE mobilization is poorly understood, however, we hypothesize that microbial secondary metabolites (such as siderophores) are involved in the mobilization process. We therefore have analyzed the genomes and metabolomes of Zn/Cd mobilizing actinobacterial strains to further elucidate the mechanisms involved in TE mobilization and plant uptake.

**FEMS-1021****Plant microbe interactions Offered by FEMS Microbiology Ecology****Back to the roots: microbiology and chemistry at the root-soil interface**

J. Raaijmakers<sup>1</sup>

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**Back to the Roots: microbiology and chemistry at the root-soil interface**

Plant roots are colonized by an astounding number of (micro)organisms that can reach cell densities much greater than the number of plant cells. Many studies have shown that members of the rhizosphere microbiome can have profound effects on seed germination, seedling vigour, plant growth and development, nutrition and tolerance to pests, diseases and abiotic stress. For the vast majority of rhizosphere microorganisms, however, there is limited knowledge on the mechanisms involved in modulation of plant growth and plant health. Here, new results are presented on how rhizosphere bacteria and fungi impact on root architecture, root growth and plant tolerance to infections by soil-borne pathogens. For the rhizosphere bacteria, results showed that representatives of the  $\gamma$ -Proteobacteria protect plants from pathogen infection by the production of chlorinated peptides and alter root architecture and plant growth via modulation of the plants' sulfur metabolism. For the fungi-plant interactions, our results showed that specific soil-borne pathogenic fungi induce changes in root architecture and plant growth via volatile organic compounds. An overview will be given on the wealth of yet unknown functions and metabolic potential of the rhizosphere microbiome.



**FEMS-2375**

**Plant microbe interactions Offered by FEMS Microbiology Ecology**

**Plant-fungal communication during arbuscular mycorrhizal symbiosis**

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Fungi from the Phylum Glomeromycota establish one of the most ancient associations with plant roots helping plants to thrive and survive under conditions of mineral deprivation. These fungi are particularly good at improving plant phosphate nutrition while in return, plants provide fungi with carbohydrates. This mutualistic symbiosis is characterized by the formation of fungal specialized structures in the cortex, arbuscules, key to the nutrient exchange and giving name to the association: arbuscular mycorrhizal (AM) symbiosis. AM fungi, like all biotrophic fungi, are committed to keep plant defenses at bay and prevent the mutualistic association to become parasitic. It is only recently that the mechanisms of how AM fungi overcome the surveillance system of the plant and manipulate their metabolism are starting to be understood. In general AM fungi use at least three mechanisms to achieve that: i) avoiding being recognized; ii) suppressing defense responses elicited after recognition iii) using a specific dialogue that allows manipulation of the plant cell program. We were first to show that similar to pathogenic microorganisms, AM fungi do have and are able to deliver effector proteins to the plant to counteract MAMP-triggered immune responses (Kloppholz et al., 2011). With the recent outcome of the first genome of an AM fungus, the effector repertoire is starting to be envisaged, and the presence of LysM containing proteins, Crinkler-like effectors and secreted proteins with NLS and DNA modification motifs points out that the similarities with pathogenic fungi are likely to be more than previously anticipated. The aim of our work is to investigate how plant roots and arbuscular mycorrhizal fungi (AMF) perceive signals from each other and establish the molecular dialogue that permits a long-lived and harmonious symbiosis.

**FEMS-2847**

**Horizontal gene transfer, evolution**

**Horizontal gene transfer, antibiotics and stress response connections**

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We have reported direct connections between the SOS response activation and HGT mechanisms such as conjugation and natural transformation, but also with gene capture in integrons. We also found that most antibiotics at sub-inhibitory concentrations induce the SOS response in *Vibrio cholerae*, and in other Gram-negative pathogens such as *Klebsiella pneumoniae*. This coupling enhances the potential for cassette swapping and capture in cells undergoing stress, while freezing the cassette arrangement in steady environments, but it also favors any mechanisms leading to resistance development through mutation. We have now undertaken the characterization of the different partners and pathways that are involved in these connections, in order to better understand these physiological responses and propose innovative strategies against the rise of resistance.

**FEMS-2871**

**Horizontal gene transfer, evolution**

**Horizontal gene transfer, microbial evolution and host adaptation**

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Gram-negative bacterial cells show copious evidence of evolution through the horizontal acquisition of novel genetic sequences with a high A+T content. In *Salmonella enterica* serovar Typhimurium, the majority of the main virulence genes fit this profile. These genes allow the bacterium to gain access to privileged sites in the host, such as the interior of epithelial cells, or to avoid being killed by components of the host defences such as macrophage. Understanding how such horizontally acquired genes are integrated into the regulatory regime of the bacterium presents a major challenge. We will provide evidence that in the case of *S. Typhimurium* regulatory integration has been achieved through combining horizontally acquired regulatory genes with regulators encoded by the ancestral genome with both operating on the virulence genes through a mechanism that exploits the special structural characteristics of A+T-rich B-DNA.

**FEMS-2851**

**Horizontal gene transfer, evolution**

**Phylogenomic transduction networks reveal genetic barriers for phage-mediated lateral gene transfer**

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Viruses that infect bacteria – termed phages – are recognized vectors of lateral gene transfer between microbial cells. Bacteria and their parasitic phages are co-evolving in a constant arms race, yet their interaction may include also mutualistic aspects. The beneficial contribution of phage-mediated gene transfer to the host fitness has been documented in diverse species and environments. Here we study the contribution of phage-mediated gene transfer to the lateral component of microbial evolution using phylogenomic transduction networks. The networks are composed of donors, phages, and recipients that are connected by 23,014 lateral gene transfer events reconstructed from genomic data. Large-scale structural differences among the stable and transient sub-networks reveal the differential contribution of temperate and lytic phage-bacteria interactions to transduction dynamics in Nature. Our evolutionary reconstruction reveals high phage-host specificity in stable interactions, while transient interactions may occur with multiple hosts. Edge weight distribution in the network suggests that gene uptake from transient hosts into the phage genome typically includes a single gene, while gene transfer into stable hosts usually comprises several genes. Yet, transient interactions constitute an important contribution to the global network structure by connecting among clusters of stable hosts. Self-donor recipients constitute a significant portion of the network (21%), implicating transduction as an important gene duplication mechanism during microbial evolution. Furthermore, donors and recipients that have a similar genome sequence and similar codon-usage are more highly connected by transduction events than the expected by chance. This indicates that donor-recipient whole genome similarity is an important factor that shapes the transduction network connectivity pattern. Our results demonstrate the implication of phage-bacteria co-evolution to phage-mediated gene transfer during microbial evolution.

**FEMS-2311**

**Horizontal gene transfer, evolution**

**Co-transfer of hypermutagenesis and symbiotic genes drives the evolution of legume endosymbionts**

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Horizontal gene transfer (HGT) is an important mode of adaptation and diversification of prokaryotes and eukaryotes and a major event underlying the emergence of bacterial pathogens and mutualists. Yet it remains unclear how complex phenotypic traits such as the ability to fix nitrogen with legumes have successfully spread over large phylogenetic distances.

To get an insight into the evolutionary mechanisms that facilitated rhizobium diversification, we experimentally replayed the evolution of rhizobia. Following introduction of the symbiotic plasmid of *Cupriavidus taiwanensis*, the *Mimosa* symbiont, into pathogenic *Ralstonia solanacearum* we challenged transconjugants to become *Mimosa* symbionts through serial plant-bacteria co-cultures.

Using whole genome sequencing we showed that co-transfer of *imuABC* error-prone DNA polymerase genes with key symbiotic genes accelerates the evolution of a soil bacterium into a legume symbiont. We demonstrate that a mutagenesis *imuABC* cassette encoded on the *C. taiwanensis* symbiotic plasmid triggered a transient hypermutability stage in *R. solanacearum* transconjugants that occurred before the cells entered the plant. The generated burst in genetic diversity accelerated symbiotic adaptation of the recipient genome under plant selection pressure, presumably by improving the exploration of the fitness landscape. Finally, we show that plasmid *imuABC* cassettes are over-represented in rhizobial lineages harboring symbiotic plasmids. Our findings shed light on a mechanism that may have facilitated the dissemination of symbiotic competency among  $\alpha$ - and  $\beta$ -proteobacteria *in natura* and provide evidence for the positive role of environment-induced mutagenesis in the acquisition of a complex lifestyle trait.

## FEMS-2924

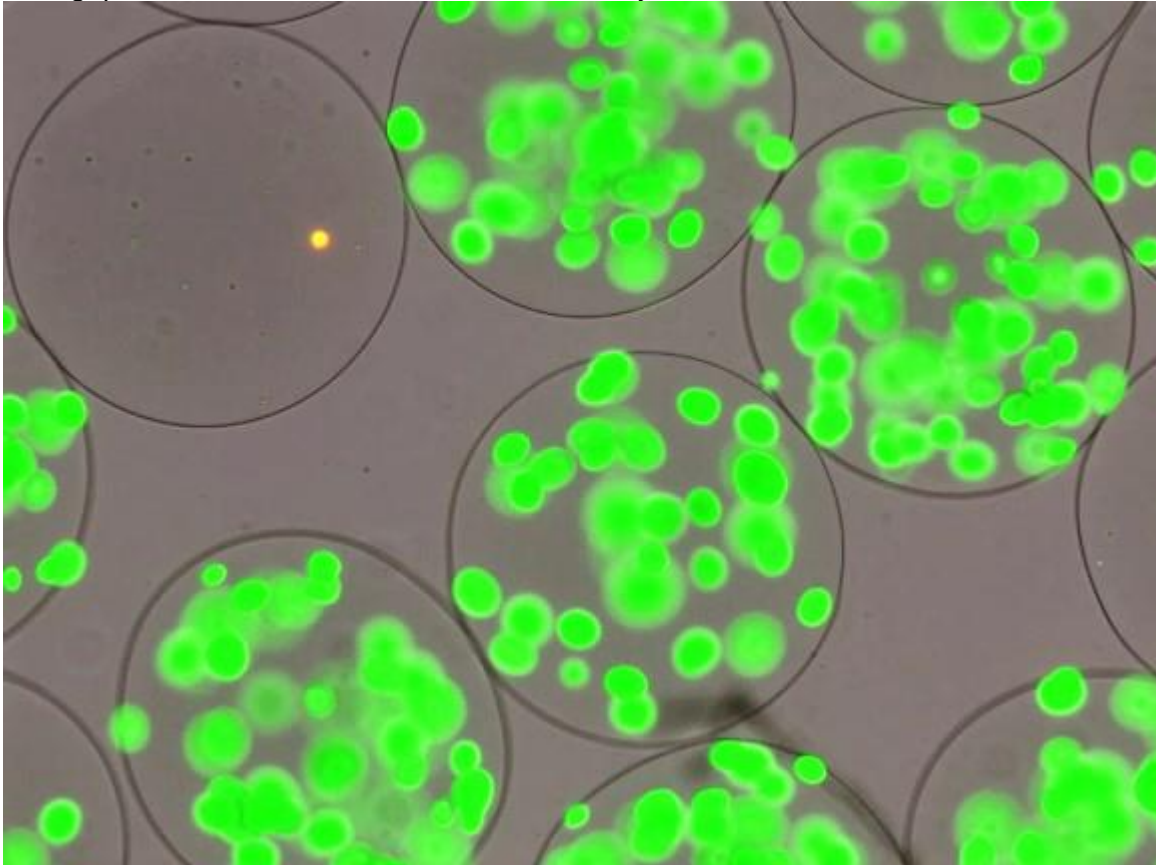
### High throughput screening technologies

#### Nano-liter reactor technology for whole cell biocatalyst optimization and discovery

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Assays for activity assessment of genetically diverse libraries are generally done in three phases, i.e. seed, growth, and analysis. Each of these phases features a considerable number of manipulation steps (e.g. inoculation, addition of reagents) and the implicit need for a strict control of the environment (e.g. temperature, humidity, sterility). This complex workflow renders the analysis of libraries in high throughput mode a rather cumbersome and costly task



We employ nL-sized gel-carriers (nanoliter reactors or NLRs) kept in suspension arrays as growth and reaction compartments. Single library cells are embedded into NLRs and proliferated to microcolonies of a few 1'000 cells required for increase of the signal-to-noise ratio. The phenotype (e.g. biocatalyst productivity) is indicated by genetically encoded or chemical fluorescence reporters. Putative positive strains are

detected on the basis of the reporter signals and isolated by an appropriate technique such as large particle flow-cytometers.

We will provide a detailed overview of the technical elements required for nLR-screenings and present two application examples: *First*, screening for antibacterial peptides secreted by *Lactobacillus* libraries at rates of 1E5 / day. *Second*, identification of *Bacillus subtilis* strains catalyzing the conversion of cellobiose to the industrial product vitamin B2 at an elevated yield.

Both protocols are used on a regular basis. While the peptide libraries screens are performed by us, the B2 protocol has successfully been transferred to a professional environment thereby further accentuating the robustness of NLR technology.

## **FEMS-2910**

### **High throughput screening technologies**

#### **Creating the Facebook for Molecular Microbial Analysis**

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The chemical make-up of microbiology is incredibly complex. It is already difficult to analyze the molecular make up of one organism, let alone 100s or 1000s of different organisms found in microbial communities? How can we look at the molecular make-up of all organisms at once? How many molecules do they have in common? How many are different? How do we retain the collective mass spectrometry knowledge of the community? As mass spectrometers are becoming faster and more sensitive we can detect a lot of molecular information. There are now mass spectrometers that can analyze 10,000 samples a day. However, there is no infrastructure to analyze this amount of molecular information or to correlate this information to other Big Data generating approaches such as sequencing. On average, however, only 1-5% of all the molecular information that is collected by mass spectrometry can be annotated. It is simply too much information for one person or lab to analyze this information with the existing tools that are available. In this lecture we will explore the strategies for organizing and visualizing the massive amount of information. Topics such as molecular networking, crowd source molecular analysis, 3D topographical mapping, MS/MS rarefaction, large scale mass spectrometry based genome mining, cross correlative analysis with molecular networking and pattern based genome mining will be covered.



**FEMS-2382**

## **High throughput screening technologies**

### **Searching for de novo induced bioactive metabolites in microbial interactions : a combined MS-based metabolomics and microNMR approach**

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Fungi possess inducible pathways that are activated in responses to stress that can produce highly bioactive defense compounds upon elicitation that need to be identified and investigated further for an evaluation of their biological properties and for a fundamental understanding of the chemical events that trigger various interactions between microorganisms. Metabolomics represents an ideal approach to highlight biomarker induction in such complex extracts. In this context the confrontation of various strains of human and plant pathogen fungi have been studied by an MS-based metabolomics approach that take advantages of UHPLC-TOF-MS fingerprinting and high resolution metabolite profiling for an efficient localization of stress-induced biomarkers. New chemometric algorithms that serve at the detection of MS features in complex mixture of fungal metabolomes have been devised. The *de novo* structure identification of novel stress-induced biomarkers was assured by subsequent LC-MS targeted microisolation and microflow NMR analyses. Various sampling, MS-based and data mining strategies were compared to highlight at best *de novo* metabolite stress-induction at the confrontation zone between microorganisms. Comparison of replicates obtained from miniaturized solid media co-culture produced in the 12-well plate format were found to be the best compromise for generating reproducible series of samples for further metabolite profiling. In some of the co-culture studied novel sulfated polyketides were evidenced in others quinonic pigments were highlighted to be strongly induced. No clear correlation between confrontation morphological patterns and metabolite induction could be made. In all cases however significant induction mechanisms were evidenced at the molecular level thanks to the advanced data mining approaches applied. *De novo* structure identification of the targeted metabolite was performed by HRMS and micro NMR after careful sample enrichment and chromatographic gradient transfer and MS-directed purification.

**FEMS-1960**

**Cell polarity and virulence**

***Brucella abortus* cell cycle in culture and inside host cells**

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*Brucellae* are responsible for a worldwide zoonosis called brucellosis. These bacteria are able to invade a large range of host cell types. The intracellular trafficking of these bacteria is characterized by two successive steps: a first step in the endosomal compartments, where the bacteria do not grow, and a second step during which the bacteria proliferate massively, usually in the endoplasmic reticulum. Using fluorescent reporters for the monitoring of growth and chromosomal segregation at the single cell level, we showed that the cell cycle of *Brucella abortus* is blocked at the G1 stage during infection of HeLa cells and RAW 264.7 macrophages. This blockage occurs during the first stage of the infection. The cell cycle is resumed at the end of this first trafficking step, when bacteria are still in the endosomal compartments. These data show that cell cycle progression is coordinated with the intracellular trafficking of the bacterium. We thus investigated the role of a conserved transcription factor and regulator of cell cycle progression called CtrA. The predicted and experimental (ChIP-seq) CtrA regulons are consistent with a major role of CtrA for the control of cell growth, cell division and DNA methylation. Interestingly, CtrA is also proposed to control the segregation of chromosome II. Indeed, the *B. abortus* genome is split in two chromosomes, the segregation of newly replicated chromosome I origins occurring before the segregation of chromosome II origins. We propose that *B. abortus*, similarly to the model bacterium *Caulobacter crescentus*, is characterized by ordered cell cycle processes, such as pole maturation and successive initiations of chromosomal replication.

**FEMS-2386**

**Cell polarity and virulence**

**Cellular polarity and attachment in the plant pathogen *Agrobacterium tumefaciens***

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*Agrobacterium tumefaciens* is best known for its ability to genetically modify plants through transfer and integration of a segment of DNA (T-DNA) into the plant genome, causing the disease called crown gall. Despite extensive studies of T-DNA transfer, little is known regarding the initial processes at the plant surface. *A. tumefaciens* forms dense biofilms on both abiotic and biotic surfaces which may increase the overall efficiency of transformation while affording protection against host defense responses. One of the primary factors mediating surface attachment in *A. tumefaciens* is called the unipolar polysaccharide (UPP), an adhesion produced at a single pole of the cell following surface contact. The UPP is one of several polarly localized cellular structures reported in *A. tumefaciens* including a tuft of unipolar flagella, and components of the Type IV secretion system (T4SS) that exports T-DNA. The cellular asymmetry is even more profoundly reflected in a polar mode of cell division for *A. tumefaciens*, in which cells multiply by budding and utilize a zonal mechanism of cell wall growth. Surface-attached *A. tumefaciens* cells generate daughter cells from their unattached pole, from which the flagella are also elaborated. The cellular asymmetry is under the control of a complex regulatory cascade designated the Control of Division and Development (CDD) pathway. Several related sensor kinases (PleC, DivJ, PdhS1 and PdhS2) and their cognate response regulators (DivK and PleD) converge on control of the CckA-ChpT-CpdR/CtrA CDD central circuit, and provide cell pole-specific modulation of division and polar development. UPP targeting to the old pole of the target cell is dependent on the PodJ protein, thought to function as a localization factor, whereas T4SS components concentrate at the pole independently of PodJ.

**FEMS-2829**

**Cell polarity and virulence**

**The long and the short of it: heterogeneity and asymmetric growth in mycobacteria**

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*Mycobacterium tuberculosis* infects billions of people worldwide and kills more than 1.5 million per year. TB remains extremely difficult to treat with antibiotics, requiring months to years of therapy for cure. The variable course of disease and treatment response suggests that functionally heterogeneous populations of mycobacteria respond differently stress. Using a quantitative single-cell approach, we show that mycobacteria deterministically generate diversity in their growth characteristics through an asymmetric growth pattern. Coupled with a cell cycle regulated by time and not size, this asymmetry creates subpopulations of cells with distinct growth rates and cell sizes that are differentially susceptible to clinically relevant classes of antibiotics. Thus, the growth pattern intrinsic to mycobacteria deterministically creates a diverse population structure that may underlie phenotypes previously thought to be controlled by external stressors. We have also observed variation among microcolonies in antibiotic susceptibility that cannot be explained by growth pole age. Armed with new reporters of cell state and mathematical models, we seek to quantitatively characterize mycobacterial subpopulations with distinct behaviors arising from asymmetric growth and division.

**FEMS-0589**

**Cell polarity and virulence**

**It takes two to tango - the interplay between two nanomachines drives horizontal gene transfer in *Vibrio cholerae***

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*Vibrio cholerae* is considered an important model organism for elucidating virulence regulation of facultative pathogens. In this context, the involvement of quorum sensing (QS) and of the type VI secretion system (T6SS) have been extensively studied even though the T6SS is been considered to be “silent” under laboratory conditions in pandemic *V. cholerae* O1 El Tor strains. Much less is known about the bacterium’s lifestyle in its natural environment, where it often associates with small crustaceans and their chitinous surfaces.

Here, we show that the chitin- and QS-dependent competence regulon of diverse pandemic and epidemic *V. cholerae* strains includes the T6SS-encoding gene clusters and that the T6SS contributes to enhanced horizontal gene transfer by means of natural transformation. We used live cell imaging to visualize components of the two competence-induced nanomachines of *V. cholerae*, namely the DNA uptake complex and the T6SS. Notably, such live-cell time-lapse imaging revealed that competence-mediated induction of the T6SS enhances horizontal gene transfer by deliberately killing neighboring non-immune cells and taking up their DNA via the competence-induced DNA uptake machinery.

**FEMS-2863**

**FEMS-ASM Joint Symposium: Polymicrobial biofilms**

**Experimental mixed species biofilms: A novel approach to evaluating interactions at a community level**

S. Kjelleberg<sup>1</sup>

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FEMS Conference presentation

Speaker: Prof. Staffan Kjelleberg

Abstract title: Experimental mixed species biofilms: A novel approach to evaluating interactions at a community level

Our understanding of the mechanisms of biofilm development has largely been derived from studies on biofilm populations, yet biofilms predominantly exist as communities. This lack of investigation into multispecies consortia reflects the relative ease by which the biofilm life cycle can be experimentally interrogated for single species systems and the difficulty to maintain experimentally tractable biofilm community systems. We have employed experimental mixed species biofilm communities to bridge this gap, also allowing us to explore mechanisms in more ecologically relevant systems and test theories from eukaryote ecology. The biofilm life cycle of two biofilm community systems – a defined three species biofilm community and a phylogenetically highly diverse biofilm granulation system – will be addressed. For the former, the establishment of a mixed defined community of *Pseudomonas aeruginosa*, *Pseudomonas protegens*, and *Klebsiella pneumonia*, has been employed to demonstrate community specific contributions to biofilm development, resistance to chemicals and predation, and variant formation by the individual member species. These findings report on mechanisms that regulate cross species interactions but which are not available for monospecies biofilms formed by either of the three members. The second experimental biofilm community system, exploring the life cycle of high species diversity granular biofilms reveals that quorum sensing signaling is a distinct community regulatory process. Here, signal producers and degraders are phylogenetically diverse and dissimilar, while a minority of the community members are capable of entertaining both signaling synthesis and quenching, as is the case for our understanding of QS signaling biology in population based model biofilms. Crucially, the granular biofilm life cycle is driven by differentiated community signaling that reflects community shifts in phylogeny, signaling synthesis and quenching.

**FEMS-2861**

**FEMS-ASM Joint Symposium: Polymicrobial biofilms**

**Clinical Implications of Biofilm-Associated Polymicrobial Infections**

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The clinical significance of microbial interactions in a host particularly those between opportunistic pathogens such as *Candida albicans* and *Staphylococcus aureus* remains largely understudied mainly due to lack of suitable animal models. To that end, we developed mouse models of co-infection to investigate the clinical implications of mixed species biofilms on host tissue and implanted catheters. In the first model, animals were orally infected with *C. albicans* and following development of oral candidiasis animals were exposed to *S. aureus* through drinking water. Findings from this model demonstrated that the extensive tissue damage caused by invading *C. albicans* hyphae provided an inlet for the typically non-invasive *S. aureus* to disseminate to the kidneys causing high morbidity and mortality. Additionally, a mouse subcutaneous catheter model was used to study *in vivo*-grown biofilms where co-infected catheter fragments were implanted subcutaneously in the animals. Following 6 days, analysis of explanted catheters revealed the formation of a thick biofilm within the catheter lumen consisting of both species embedded in a matrix. Further analysis indicated increase in *S. aureus* produced eDNA in mixed biofilms and enhanced tolerance of *S. aureus* to vancomycin. Combined the findings from these studies demonstrated that the onset of oral candidiasis may constitute a risk factor for disseminated bacterial disease. Further the co-adherence of *C. albicans* and *S. aureus* to abiotic surfaces results in formation of resistant biofilms on indwelling medical devices. These novel findings demonstrate the gravity of *C. albicans*-*S. aureus* interactions in the host, warranting awareness in terms of prophylactic therapeutic measures.

**FEMS-2377**

**FEMS-ASM Joint Symposium: Polymicrobial biofilms**

**Candida albicans-Staphylococcus aureus intra-abdominal infections: at the crossroads of inter-Kingdom virulence and host response**

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The polymorphic fungus *Candida albicans* and the ubiquitous bacterial pathogen *Staphylococcus aureus* are common co-infecting microbes during intra-abdominal infections (IA), often resulting in high morbidity and mortality. Using a murine model of IAI, our previous work demonstrated that synergistic host inflammation (specifically, prostaglandin E2) is associated with mortality. However, the microbial virulence factors contributing to infectious synergism remain undefined.

Previous studies suggested that staphylococcal toxins were associated with poor outcome during murine IAI. Thus, we hypothesized that *C. albicans* may augment the expression of *S. aureus* toxins during co-infection. To test this hypothesis, cultures of *S. aureus*, *C. albicans*, or both organisms together were grown under planktonic or biofilm growth conditions. Cell-free supernatants from polymicrobial cultures showed increased hemolysis *in vitro*. Use of an isogenic *S. aureus*  $\Delta agrA$  mutant confirmed that enhanced hemolysin production was *agrA*-dependent. Use of a GFP-reporter plasmid demonstrated enhanced *agr* activity in co-culture as compared to monomicrobial growth, and qPCR and Western blot analysis of *agr*-regulated genes further supported these results. Lastly, mice were intraperitoneally inoculated with sub-lethal doses of *C. albicans*+*S. aureus*, *C. albicans*+*S. aureus* ( $\Delta agrA$ ), or *S. aureus* alone and monitored for morbidity and mortality. Alarming, 80% of the mice co-inoculated with *C. albicans*+*S. aureus* (WT) succumbed by d 1 post-inoculation, while only 30% of the mice co-inoculated with *C. albicans*+ $\Delta agrA$  succumbed to the infection (d 8 post-inoculation). All mice inoculated with JE2 alone survived throughout the infection time course. Furthermore, assessment of  $\alpha$ -toxin in the lavage fluid by immunoblot and ELISA confirmed increased toxin during co-infection, despite similar bacterial burden as monomicrobial infection.

In summary, *C. albicans* can augment *S. aureus* toxin production, potentially explaining the devastating pathology of fungal-bacterial IAI.



**FEMS-1161**

**FEMS-ASM Joint Symposium: Polymicrobial biofilms**

**Contribution of bacterial volatile molecules to the dynamics of polymicrobial bacterial biofilm communities**

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Bacteria produce and sense a high diversity of signals and cues to adapt their behaviors to changing environments. Among them, volatile compounds produced by fungi and plants were shown to modify bacterial phenotypes and activities. However, besides being perceived as odors, attractive scents or pollutants, the influence of bacterial volatile molecules on bacterial biology itself remained poorly explored. I will discuss how some bacterial volatile compounds diffusing in heterogeneous environments, such as soil, organic tissues and microbial mats can mediate airborne chemical interferences and contribute to shape polymicrobial bacterial communities.

**FEMS-0996**

**Microbiome and human health Organized by EAM**

**An evolutionary view of the Holobiont theory and its implications for host development**

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The mammalian microbiome is formed out of a maternal hand-over of microbes, diet and lifestyle that will influence host physiology throughout life. Moreover, microbes and its host also share, in part, their biological and biochemical needs. This assumption forms the basis of the Holobiont theory. This theory of evolution proposes that the object of natural adaptation and selection is not the host (its genes and organs), but the host together with its microbiome (all genes and its metabolites) - The Holobiont. When the host – The Holobiont - is challenged by dramatic changes, such as alteration in dietary intake, malnutrition, reduced physical activity or unwanted severe stress, a Holobiont will employ strategies and adaptation mechanisms to handle these changes including signals transmitted to the microbiome community. The microbiome in turn, will reciprocate and transmit signals to its host. The last decades of reductionist research aiming to decipher the underlying mechanisms responsible for the epidemic development of life style related diseases, by use of Genome Wide Association Studies has not considered the Holobiont perspective and consequently, the adaptation strategies developed by the microbiome and its signals transmitted to the host has not been included in the analysis. In my presentation I will discuss the Holobiont concept and present data which support how a host responds to this bilateral microbiome-host communication related to development and metabolic homeostasis.

**FEMS-3161**

**Microbiome and human health Organized by EAM**

**Gut Microbiota and Type 2 Diabetes**

F. Bäckhed<sup>1</sup>

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The human gut is inhabited with trillions of bacteria, gut microbiota, that have co-evolved with us and affect our physiology within and outside the gut. The gut microbiota has recently been suggested as a novel contributor to obesity and related comorbidities, such as type 2 diabetes (T2D) and cardiovascular diseases (CVD). We recently found that the gut microbiota is altered in patients with CVD and T2D and that we can classify patients and T2D patients based on the microbiota. Using germ-free mice we have causally linked the gut microbiota to obesity and insulin resistance and have recently found that the gut microbiota modulates adipose inflammation, bile acid signaling, and enteroendocrine cell function. However, the underlying mechanisms(s) by which the gut microbiota induces signalling is yet to be dissected. By combining defined microbial communities, multi-omics approaches with genetically modified mice we are beginning to clarify host microbial interactions and their metabolic responses.

**FEMS-2795****The Species Concept in the Genomic Era Supported by the Bergey's Manual Trust and Organized by ICSP****Genome sequences as nomenclatural type material for novel taxa**

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Major goals of the Bacteriological Code are to provide all prokaryotic species with a single name and insure that each name refers to a single species. Currently, these goals are achieved in part through designation of type strains, which are viable cultures deposited in two public culture collections. These cultures then become the reference material for comparison to the type strains of other validly described species.

The genome sequence provides sufficient information to satisfy the goals of the Bacteriological Code for type material. Based upon genome sequence similarities, it is possible to determine if two strains are members of the same species. The genome sequence also provides sufficient information about the growth properties, physiology, metabolism, and lifestyle to support polyphasic classifications. Thus, the Bacteriological Code should be modified so that genome sequences from clonal populations or single cells are acceptable type materials upon deposition of DNA to insure authenticity.

This change will allow naming of taxa not readily cultivated by service culture collections and uncultured taxa. Moreover, the current system was developed when the number of type strains was in the thousands. The number of type strains is now greater than 12,000 and continues to increase. Culture collections are unfairly burdened with the task of maintaining and distributing this large number of strains. Allowing genome sequences to serve as type material will allow culture collections to reallocate resources to the most important and valuable cultures.

**FEMS-2796**

**The Species Concept in the Genomic Era Supported by the Bergey's Manual Trust and Organized by ICSP**

**Applying an evolution-based species concept for the delineation of bacterial species: The *Pantoea* example**

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Bacterial species are commonly defined by applying a set of predetermined criteria, such as DNA:DNA hybridization values, 16S rRNA sequence similarity and phenotypic and chemotaxonomic comparisons. Alternative genome-based similarity criteria have also been proposed to define bacterial taxa. We have previously shown that the metrics based on genome data support all of the validly described *Pantoea* species. However, the question remained whether the same species would have been recognised when taxa were delineated using an evolution-based species concept instead of fixed or quantitative criteria. Here we utilized *Pantoea* and the available genome data to investigate whether an evolution-based concept, in which species represent unique and genealogically exclusive groups of isolates on phylogenetic trees, would allow for delineation of the known species.

The core genomes (genes present amongst all member of the group) for *Pantoea* species were determined. These genes were subjected to phylogenetic analyses as they collectively represent the dominant evolutionary signal that depicts vertical descent. The core gene content was also characterized in terms of general cellular functions.

The *Pantoea* core genome consisted of approximately 2500 genes and differed markedly from the genomes of other closely related genera. Similar phylogenies were inferred for the various functional sets of genes. Although slight changes in sister relationships were observed for some of the species, the examined *Pantoea* species all represented exclusive genealogical groups. Our results thus indicated that a genome-based comparative and evolutionary approach could be a preferred alternative to fixed criteria when delineating coherent bacterial taxa.

**FEMS-2356**

**The Species Concept in the Genomic Era Supported by the Bergey's Manual Trust and Organized by ICSP**

**Towards a harmonized classification embracing cultured and uncultured taxa**

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**Background:**

Molecular tools applied to understand microbial diversity have evidenced the very large extent of undescribed taxa. The estimated richness exceeds in orders of magnitude the current ~12,000 classified species. However, there is a need to find faster ways to catalogue the biosphere's diversity, and try to overcome the major problem in classification that is bringing the type material into pure culture.

**Objective:**

For this study we have measured the extent of hitherto discovered putative taxa in 16S rRNA gene databases using taxonomic thresholds that have been previously calculated with all cultured *Bacteria* and *Archaea*. In addition, we have evaluated the current species definition and its application to recognize uncultured microbial populations thriving in natural environments and detected through metagenomic approaches.

**Conclusions:**

The current species definition for prokaryotes is strongly based on its circumscription by genome sequence comparisons and genetic distances of housekeeping genes, together with a discriminative phenotypic property. The set of environmental 16S rRNA gene sequences in public repositories matched about 250,000 species, 80,000 genera and 1,300 phyla, and the predicted total number of species in the biosphere ranges from  $10^6$  –  $10^7$ . In addition, the new sequencing technologies allow retrieving individual population's (meta)genomes of uncultured organisms that can be used to understand their phylogeny and genomic uniqueness, and predict their metabolism as for cultured organisms. This will allow implementing metagenomics into the *Candidatus* proposals of organisms not showing conspicuous prominent characters, and speed up the description of uncultured organisms in a similar way to those in pure culture.

## FEMS-2708

### The Species Concept in the Genomic Era Supported by the Bergey's Manual Trust and Organized by ICSP

#### Type species in the genomic era of Mollicutes

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Since its inception, the International Code of Nomenclature of Bacteria has emphasized the importance of type material as a reference to be used when considering the identity of specimens. Nomenclatural types also permanently bear the name of the taxon. Investigators expect that types should exemplify the defining characteristics of the taxon as it exists within a hierarchy of superior taxa. The Code is sufficiently flexible to allow that types later realized to be unsuitable can be replaced with neotypes as knowledge advances.

The type of genus *Mycoplasma*, hence the *Mycoplasmataceae*, *Mycoplasmatales* and class *Mollicutes* is *Mycoplasma mycoides* subsp. *mycoides* strain PG-1<sup>T</sup>. Modern phylogenetic analyses situate *M. mycoides* in the distant order *Entomoplasmatales*, so if discovered today PG-1 would be doubtfully referred to *Mycoplasma*. Our objective was to explore genomic methods that might be used to identify candidates for neotype of the genus.

A well-chosen neotype might be expected to exhibit genomic consistency with others in its genus. JSpecies was used to calculate ANI values by pairwise comparisons of the complete genome sequences of 20 species representing six families within class *Mollicutes*. ANIm was calculated by the NUCmer algorithm of MUMmer and ANIb was calculated by reciprocal BLASTN. The alignment-free tetranucleotide frequency correlation coefficient was also calculated. The matrices were visualized as unrooted trees using PHYLIP, then reviewed by the ICSP Subcommittee on the taxonomy of *Mollicutes*. The Subcommittee unanimously agreed that the genomic trees were not congruent with the shared view of the taxonomic structure of the class. Although evidently not suitable for determination of supraspecific relationships, genomic analyses remain valuable for species-level circumscription of mollicutes.

Reference: **Firrao, G. & D. R. Brown (2013).** *Int J Syst Evol Microbiol* **63**, 2361–2364.

## **FEMS-2244**

### **Antibiotic resistance and environment**

#### **Antibiotic resistance in the urban water cycle: origins, fate and risks**

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The urban water cycle refers to the journey of water from the collection, disinfection and purification for human consumption to its return to nature as treated wastewater. Over this cycle, water is inhabited by different bacterial populations, some strictly environmental, others of human or animal origin, most of which considered contaminants. Multidrug resistant bacteria, sometimes harboring antibiotic resistance (AR) genes of clinical relevance, are commonly found in each of these aquatic environments.

It is estimated that AR bacteria are responsible annually for > 20000 deaths, in both the US and the EU. Although these values are registered in the clinical settings, it is recognized that the environment, in particular water, plays an important role on the dissemination of AR from and to humans.

Urban wastewater, either raw or treated and ready to discharge in the environment, presents a high prevalence of AR to “old” antibiotics, suggesting the cumulative effect of this form of contamination. Moreover, almost all AR genes of clinical concern can be detected in wastewater, suggesting their rapid dissemination.

In contrast, drinking water, in principle free of pathogens, is inhabited by an impressive diversity of environmental bacteria, some of which exhibit multidrug resistance phenotypes, probably intrinsic in those species. Although not posing an acute problem of AR dissemination, intrinsically AR bacteria may have also adverse human health effects.

Nowadays, it is difficult to assess the risks of the transmission, directly or indirectly, of AR bacteria from the environment to humans. A major limitation is related with the absence of surveillance and data sharing systems comprising both environmental and clinical data, allowing a global overview of the emergence and evolution of AR and how it can affect human health.

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**FEMS-2353**

**Antibiotic resistance and environment**

**Disinfection of urban wastewater by conventional and new processes: effect on antibiotic resistance**

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The effluents of urban wastewater treatment plants (UWWTPs) are among the main point sources of antibiotic resistance pollution of surface waters. In several countries, disinfection process is the last step of UWWTPs to minimize the release of antibiotic resistant bacteria and genes (ARB&Gs) in the effluent. But in some country UWWTP effluent is disposed in the receiving water body without any disinfection treatment. Typical disinfection processes include chlorination and UV radiation but other disinfectants/oxidants such as ozone, chlorine dioxide and hydrogen peroxide are also used. Unfortunately, ARB&Gs have been detected in the effluents of UWWTPs raising the suspect that conventional disinfection processes (particularly chlorination and UV radiation) not effective in controlling antibiotic resistance spread into the environment. Therefore, in the last years non conventional disinfection technology such as that based on Advanced Oxidation Processes (AOPs) has been investigated as possible alternative to conventional disinfection processes for controlling antibiotic resistance spread.

In this work the results of disinfection experiments by chlorination, UV radiation and AOPs are summarized. The effect of disinfection processes on antibiotic resistant *E. Coli* (from the effluent of secondary treatment of an UWWTP) in terms of inactivation, bacterial regrowth and antibiotic resistance was evaluated. Moreover, the effect of an AOP (namely TiO<sub>2</sub> photocatalysis) on the mechanism of antibiotic resistance transfer was also investigated.

AOPs can be more effective than conventional disinfection processes in the inactivation of ARB but further studies on the effect of AOPs on antibiotic resistance transfer are needed to better understand the potential of residual ARB and mobile genetic elements of transferring antibiotic resistance after disinfection treatment.

## **FEMS-1376**

### **Antibiotic resistance and environment**

#### **DETERMINING THE MINIMAL SELECTIVE CONCENTRATIONS OF ANTIBACTERIAL AGENTS IN COMPLEX AQUATIC BACTERIAL COMMUNITIES**

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#### **Background**

A mixture of antibiotics and co-selective agents, such as biocides and metals, reach the environment through sewage effluents. It is unknown to what extent antibacterial agents select and co-select for resistance in aquatic ecosystems e.g. wastewater treatment plants and receiving waters.

#### **Objectives**

The aim was to determine the minimal selective concentration (MSC) of an antibiotic (tetracycline) and the minimal co-selective concentrations (MCCs) of a common biocide (cetyl trimethyl ammonium chloride; CTAC) in complex aquatic bacterial communities.

#### **Methods**

Treated sewage effluent (Gothenburg, Sweden) was diluted and used as inoculum to establish biofilms in flow-through systems containing different concentrations of antibacterial agents. Biofilms were screened for their resistance/tolerance using selective plating, characterization of isolates, metagenomics analyses (resistance genes and taxonomic composition), and pollution induced community tolerance (PICT).

#### **Conclusions**

Concentration-response curves for tetracycline were obtained for selective plating, *tet*-genes and PICT. Based on selective plating the MSC of tetracycline was 10ng/mL ( $p=0.009$ ), which correlates well with an increased MIC<sub>50</sub>-value (concentration inhibiting 50% of the isolates). Increases of other resistance genes, ISCR2 and *int1* were observed with increasing tetracycline concentrations. Effects of tetracycline on taxonomic composition are under evaluation.

CTAC selects for self-tolerance and co-tolerance to chloramphenicol, tetracycline and erythromycin. The MCCs of CTAC as well as effects on resistance gene frequencies and taxonomy remain to be evaluated.

Knowledge of the selective and co-selective properties of tetracycline and CTAC generated here will facilitate the establishment of safe emission limits. This could guide actions to reduce risks for resistance selection in the environment.

**FEMS-1789**

**Antibiotic resistance and environment**

**EXPLORING PLASMID-BASED DISSEMINATION OF ANTIBIOTIC RESISTANCE GENES IN ENVIRONMENTAL MATRICES**

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**Background**

Plasmids are important actors of antibiotic resistance gene dissemination that contribute to reduce the effectiveness of our therapeutic potential.

**Objectives**

Our objectives are to identify permissive environments and/or conditions driving plasmid transfer in microbial communities.

**Methods**

Lately, we developed a sensitive molecular approach based on qPCR allowing the detection of rare transfer events of known plasmids in natural microbial communities. Basically, it consists in inoculating microcosms with a donor bacterium and to quantify the relative abundance of both the plasmid and the initial host DNAs over time. Since conjugative transfer is an intercellular mode of DNA replication, the plasmid to donor DNA ratio increases in the community DNA when the plasmid transfers into the indigenous population.

**Conclusions**

The transfer of the broad host range IncP-1 $\beta$  plasmid pB10 was studied in various environmental matrices. Under low level of inoculation, the transfer of pB10 in complex environments appeared relatively rare and was strongly matrix dependent. In some instance, the transfer of pB10 appeared influenced by eukaryotic predation, which alternatively could promote or inhibit plasmid transfer depending on the environmental matrix considered. An estimation of the IncP-1 $\alpha/\beta$  plasmids abundance by qPCR demonstrated that pB10 transfer tends to be supported by environmental matrices exhibiting a higher content of IncP-1 $\alpha/\beta$  plasmids. This tend to show that the relative abundance of IncP-1 plasmids in a given microbial community reflects its permissiveness to the transfer of plasmids belonging to the same incompatibility group, which seemed to prevail over transfer limitation due to the superinfection immunity (exclusion & incompatibility).

**FEMS-2555**

**Antibiotic resistance and environment**

**METAL SPECIFIC MODULATION OF COMMUNITY PERMISSIVENESS  
TOWARDS BROAD HOST RANGE PLASMIDS THROUGH STRESS**

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**Background**

The extent by which antibiotic resistance encoding conjugal plasmids transfer in microbial communities is of acute relevance in the age of massive antibiotic usage. The occurrence of stressors, like metals, might play a major role in altering the acute permissiveness of a bacterial community, since plasmid transfer is considered a main process in immediate stress response and adaptation to environmental changes.

**Objectives**

We evaluated: Does the introduction of metal stress alter a soil community's permissiveness towards plasmids? Is a potential stress response general or a metal specific?

**Methods**

Hence, using a [3H]-leucine incorporation approach, we measured 20% and 50% inhibition concentrations for 5 metals. A *mCherry*-tagged donor carrying the *gfp*-tagged plasmid pKJK5 was mated with a soil bacterial community and exposed to metal stress. Transconjugants were quantified and isolated using advanced microscopy and fluorescent activated cell sorting. Sorted transconjugants were analyzed by pyrosequencing.

**Conclusions**

The imposed metal stress lowered the plasmid transfer frequency in filter mating assays. The intensity of this effect was metal specific and couldn't be explained by measured growth inhibition of the recipient community because the exposure was normalized to similar inhibition. Results revealed an effect on transconjugant diversity for the heavy metals, Nickel or Copper, while Arsenic exposure caused no effect. The changes in transconjugal pool diversity couldn't be directly correlated to a change in community diversity through the introduced stress. Our results suggest that this effect is not general but corresponds to a metal specific stress response of the soil microbial community with regard to plasmid transfer.

## EXPLORATION OF THE REMOVAL POTENTIAL OF WASTEWATER ANTIBIOTIC RESISTANCE GENES BY SELECTED PHOTOCATALYTIC AND BIOLOGICAL TREATMENT TECHNOLOGIES

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### Background

The pressure applied onto bacteria in aquatic environments by the extensive use of antibiotics, leads to increased incidence of antibiotic resistance (AR) (Volkman et al., 2004), increasing the prevalence of nosocomial diseases. There is limited research regarding alternative actions of controlling AR in wastewater treatment plants (WWTPs), i.e. advanced oxidation processes (AOPs) and new biological methods which may remove AR more efficiently (Rizzo et al., 2013).

### Objectives

The aspects examined were: i) the prevalence of selected AR genes (ARGs) at different steps of the WWTP process ii) the ARGs removal efficiency (*vim*, *vanA*, *mecA* and *lak* genes) of a pilot scale Membrane BioReactor (MBR) and of two AOPs (solar photo-Fenton treatment and heterogeneous (TiO<sub>2</sub>) photocatalysis).

Step of treatment	Detector	% change (/100 ng of total DNA)	Detector	% change (/100 ng of total DNA)	Detector	% change (/100 ng of total DNA)	Detector	% change (/100 ng of total DNA)
Primary influent		-		-		-		LOD
MBR Mixed liquor Suspended Solids		28		67		69		-
MBR effluent	<i>vanA</i> Sybr	4400 (increase)	<i>lak1</i> Sybr	509 (increase)	<i>vim1</i> Sybr	18100 (increase)	<i>mecA</i> Sybr	-
Solar photo-Fenton treatment		7300 (increase)		6500 (increase)		99		-
TiO <sub>2</sub> heterogeneous photocatalysis		98		96		96		-

**Table 1.** Removal of examined antibiotic resistance genes after the investigated AOPs and biological treatment.

### Methods

The AOP experiments took place under bench-scale conditions while the MBR had a capacity of 10 m<sup>3</sup> day<sup>-1</sup>. Real-time qPCR assessment followed the filtration and DNA extraction of the samples.

### **Conclusions**

The highest prevalence of *lak* and *vanA* genes was found in solar photo-Fenton effluent. The highest *vim* prevalence was found in MBR effluent (Table 1), indicating an increase in the examined ARGs after treatment. Further optimization of the examined technologies must occur to ensure safe disposal of treated effluents into aquatic ecosystems.

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**FEMS-2798**

**Food Microbiology**

**Proteomics, thermal robustness and germination heterogeneity of *Bacillus* spores**

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Bacterial spores are ubiquitous in nature. They are stress resistant entities that can withstand high environmental temperatures, chemical insults and physical stress such as radiation or increased pressure. Spores are a concern to microbiological food stability due to these characteristics as upon survival of a preservation process they may start to germinate and grow out in food causing food spoilage. In addition germinating and outgrowing spores at undesired times and places pose a significant health burden. The challenge is amplified due to the heterogeneous germination and outgrowth behaviour of an isogenic spore population. We set out to analyse effects of thermal stress and the presence of a weak organic acid preservative on *Bacillus subtilis* spores of different maturation stages. Spore germination and outgrowth was assessed using live-imaging including the monitoring of intracellular pH with IpHluorin. Significant heterogeneity in spore germination and outgrowth was observed and monitored using the newly developed Sporetracker image analysis tool. Thermal stress clearly enhances heterogeneous germination behaviour. To analyse whether different levels of spore coat cross-linking may be involved in the (heterogeneous) stress response we set out to identify such cross-links by mass-spectrometry analysis of the insoluble coat fraction of *B. subtilis* spores of different maturation levels. Heat resistance of spores from all samples was also tested. Using our gel-free proteomic approach and LC-FTICR-MS/MS analysis we monitored the efficiency of tryptic digestion of proteins in the coat during spore maturation over a period of 10 days, using metabolically <sup>15</sup>N labelled mature spores as a reference. The results showed that during spore maturation the loss of digestion efficiency of outer coat (for instance CotG, CotC, CotU) and crust (CotY, CotZ) proteins synchronized with an increased heat resistance.



## **FEMS-2430**

### **Food Microbiology**

#### **The sweet secrets of industrial strain improvement**

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Lactic acid bacteria (LAB) are a functionally related group of Gram-positive bacteria known essentially for their roles in food bioprocessing. The successful biotechnological application of LAB depends to a great extent on their unique phenotypic traits, which among others include fast acidification of the medium, texture and flavour forming abilities, bioprotection, and health promoting properties. However, the highly dynamic food market and ever-changing preferences from end consumers demands constant focus on product development. Fostered by this market push, Chr. Hansen places continuous efforts into the development of new LAB strains and cultures with novel properties and superior performance for the dairy industry. In the 21<sup>st</sup> century, genetic engineering could make such tasks more ease to accomplish. However, the tight requirements of regulatory agencies and the negative perception by consumers of genetically modified foods impose the exclusive use of natural strain improvement methodologies. Thus, at Chr. Hansen innovative product development includes combining classical strain improvement techniques such as random mutagenesis, dominant selection, adaptive laboratorial evolution, with the insights gained from the molecular understanding of our model systems. This approach will be illustrated with relevant example cases of strain improvement for the dairy industry.

**FEMS-1218**  
**Food Microbiology**

**A MOBILE GENETIC ELEMENT IS RESPONSIBLE FOR SUBSTANTIALLY  
INCREASED HEAT RESISTANCE OF BACILLUS SUBTILIS SPORES**

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**Background**

Spores of *Bacillus subtilis* are able to survive various harsh environmental conditions, including different heat preservation treatments used in food processing. Twenty strains of *B. subtilis* could be divided into two distinct groups with different spore heat resistances where the high spore heat resistance group required 100 times longer heating at 120°C to obtain the same level of spore inactivation.

**Objectives**

The goal of this study was to identify a genomic determinant for increased spore heat resistance, and to understand the underlying molecular mechanism mediating this increase in heat resistance of spores.

**Methods**

Whole genome sequencing was performed for these food isolates of *B. subtilis*, followed by correlating the genome content to the corresponding spore heat resistance phenotype. A transposon was identified and the insertion location was verified by PCR. Transfer of this element was achieved using filter mating.

**Conclusions**

A set of genes were uniquely present in the strains producing spores with increased heat resistance, and were located on transposon. The integration site of the transposon was found in the same gene for all strains producing high heat resistant spores. We demonstrated that the transposon was directly responsible for the very high heat resistance of spores; transfer of the transposon to a strain with significantly lower heat resistance (*i.e.* *B. subtilis* 168) rendered a phenotype of high spore heat resistance. The exact molecular mechanisms that mediate the increase of spore heat resistance are not known and the role of the genes on the transposon is currently subject of further investigation.



**RECONSTRUCTION OF THE METHIONINE TO CYSTEINE CONVERSION  
PATHWAY IN LACTOBACILLUS PARACASEI FAM18149 BASED ON  
COMPARATIVE GENOMICS AND WHOLE TRANSCRIPTOME ANALYSIS**

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**Background**

The flavor of cheese results from metabolic activities of bacteria like *Lactobacillus paracasei* during cheese ripening. Among many others, volatile sulfur compounds (VSCs) such as methanethiol, methional and hydrogen sulfide are key flavor compounds. Methionine is the main sulfur source in cheese, and is probably the precursor for bacterial VSC production. We observed that various strains of *L. paracasei* grew in medium that contained methionine as the sole sulfur source. Interestingly, these strains were also capable to produce VSCs.

**Objectives**

*L. paracasei* strains that grow with methionine as sole sulfur source have the potential to be used as flavor enhancing adjunct cultures in cheese making. A better understanding of the pathways involved in the metabolism of methionine by this species is of importance for proper strain selection.

**Methods**

*L. paracasei* FAM18149 was grown in medium with cysteine as only sulfur source and in medium with methionine as only sulfur source. The transcriptome of both conditions was measured using RNA-seq. The significantly regulated genes were studied in a gene trait analysis including genomes of 23 additional strains.

**Conclusions**

We found 55 significantly regulated genes. Among the regulated genes we identified a cluster that is only present in strains that grow with cysteine as only sulfur source. The cluster consists of a cysteine synthase, a cystathionine gamma-/beta-lyase and a serine acetyltransferase.

Based on these results we were able to reconstruct the whole pathway of methionine to cysteine conversion and show that the cluster comprises the key enzymes of the pathway.

**FEMS-2204**

**Food biotechnology**

**MLST ANALYSIS TO CHARACTERIZE THE STRESS RESPONSE IN LACTOBACILLUS PARACASEI.**

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Background: The interest regarding the genetic variability among *L. casei* group members is increasing. These information are highly relevant in order to improve the knowledge and understand the correlation between genetic traits and biological properties, but also phenotypic characteristics and industrial properties of the strains. Objectives: The study of the correlation between the genetic profile of the strains and the stress response.

Methods: 25 *L. paracasei* strains were selected for MLST analysis; 8 genes related to stress response (*ctsR*, *hrcA*, *cydD*, *cydA*, *nox*, *npr*, *pox*, *dnaK*) were chosen as target genes. After the comparison of the obtained sequences, a Sequence Type (ST) number was assigned to each strain. MEGA version 6.06 software (<http://www.megasoftware.net>) was used to compare and align the sequences and phylogenetic trees were constructed using the UPMGA. DnaSP software version 5.10.1 was used to perform the descriptive analysis.

Conclusions: After PCR of the internal sequences of the selected loci (*npr*, *nox*, *hrcA*, *ctsR*, *cydA*, *cydD*, *dnaK*, and *pox*), the amplicon was obtained for all the tested strains, confirming the presence of the target genes. All the studied strains resulted having a different Sequence Type (ST). The mean G + C content of the different gene fragments was higher than 0.45, meaning that the analysed genes are almost stable. Observing the MLST UPGMA trees, the strain's cluster confirmed a correlation between the different genetic profile of the stress related genes and the different stress resistance of the strains.

**LISTERIA MONOCYTOGENES SURVIVAL STRATEGIES: POPULATION  
HETEROGENEITY AND STRESS RESISTANT VARIANTS**

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**Background**

The food-borne pathogen *Listeria monocytogenes* is a Gram-positive microaerophilic facultative anaerobic rod and the causative agent of listeriosis. This pathogen is notorious for its robustness and a range of studies have been performed that supplied insight in adaptive stress response and survival strategies.

**Objectives**

The objectives of this study are the assessment of *L. monocytogenes* population heterogeneity and the genotypic and phenotypic analysis of stress resistant variants obtained after high hydrostatic pressure, heat and acid exposure.

**Methods**

A range of phenotypes was determined for the different stress resistant variants obtained using *L. monocytogenes* EGDe, LO28 and ScottA, including level of acid resistance, growth rates at different temperatures, and biofilm-forming capacity. Whole genome sequencing analysis of *L. monocytogenes* wild types and selected stress resistant variants was performed, followed by cluster analysis of genotypic and phenotypic parameters to assess population diversity within *L. monocytogenes* strains.

**Conclusions**

Significant differences in phenotypes among variants were observed that pointed to differences in robustness and growth performance under food-relevant conditions. Whole genome sequencing analysis of *L. monocytogenes* wt and stress resistant variants revealed mutations in *ctsR*, encoding a class III heat shock repressor, and in *rpsU*, encoding ribosomal protein S21. Cluster analysis of genotypic and phenotypic parameters revealed large population diversity even within one *L. monocytogenes* strain and that different adverse conditions select for different variants. The large population diversity of *L. monocytogenes* signifies the organism's genetic flexibility, which in turn may contribute to the survival and persistence of this human pathogen in food-processing environments.

**FEMS-1969**

**Food Microbiology**

**ANTIMICROBIAL ACTIVITY AND GROUP B VITAMIN PRODUCTION OF LACTIC ACID BACTERIA ISOLATED FROM CHICHA, A TRADITIONAL MAIZE-BASED FERMENTED BEVERAGE**

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<sup>2</sup>Tecnología y Desarrollo, Centro de Referencia para Lactobacilos (CERELA), San Miguel de Tucumán, Argentina

<sup>3</sup>Istituto di Microbiologia-Centro Ricerche Biotecnologiche, Università Cattolica del Sacro Cuore, Piacenza-Cremona, Italy

**Background**

Many lactic acid bacteria strains show extraordinary metabolic abilities, which are interesting for their application in functional food development such as group B vitamin overproduction. In this context, the determination of quality and food safety related traits i.e. antibiotic resistance are required.

**Objectives**

In this study, 29 *Lactobacillus plantarum* strains isolated from *chicha*, a traditional maize-based fermented Andean beverage, were previously selected by their ability to produce folic acid. Thus, we focused on evaluating additional activities relevant to functional food industry and to food safety and quality.

**Methods**

*Lactobacillus plantarum* strains were screened for riboflavin production (by reverse phase HPLC with fluorescence detector) and for the presence of riboflavin production related genes (*ribA*, *ribB*, *ribC*, *ribG* and *ribH*), antibacterial activity (Gaudana *et al.* 2010. *Brit J Nutr.* 103: 1620–1628) and antifungal activity (Magnusson *et al.* 2003. *FEMS Microbiol Lett.* 219:129-135). Regarding food safety, antibiotics resistance was tested as recommended by EFSA (2012) for *Lb. plantarum*.

**Conclusions**

Most strains strongly inhibited the growth of *Listeria innocua* CECT 910<sup>T</sup>, *Escherichia coli* CECT 5947, *Salmonella enterica* CECT 4138 and showed antifungal activity against *Aspergillus oryzae* CECT 2094. Some of them, inhibited *Aspergillus niger* CECT 2807, as well. Among them, four strains exhibited traits of interest for the functional food industry. They produced 400ng/ml riboflavin, determined by HPLC, and showed the amplification products corresponding to genes in the riboflavin synthetic pathway. All of these were sensitive to the antibiotics tested and were able to inhibit the growth of relevant food pathogens and spoiler fungi.





**FEMS-1244**

**Molecular principles of biofilm formation and dispersion**

**Regulation of functional interferences between *Escherichia coli* type 1 fimbriae and Ag43 phase-variable adhesins**

J.M. Ghigo<sup>1</sup>

<sup>1</sup>*Department of Microbiology, Institut Pasteur, Paris, France*

Bacteria use a large number of adhesins involved in bacteria/surfaces and bacteria/bacteria interactions to colonize their environments. In *Escherichia coli*, there are two essential and prototypic adhesins: the chaperone-usher type 1 fimbriae, involved in mannose-dependent interactions to host cells, and the auto-transporter adhesin Ag43, mediating bacterial auto-aggregation. These two adhesins are regulated by two distinct phase variation mechanisms: a promoter recombination inversion for type 1 fimbriae and an epigenetic mechanism involving the Dam methylase and the oxidative stress regulator OxyR for the auto-transporter adhesin Ag43. Since expression of long type 1 fimbriae physically interferes with short Ag43 adhesins, the functions of these two adhesins are antagonistic, which raises the question of how *E. coli* coordinates their expression. I will describe previously unsuspected regulatory connection between phase variation mechanisms and stress responses and discuss how it contributes to coordinate the expression and exposition of *E. coli* surface adhesins and bacterial colonization capacity.

**FEMS-2380**

**Molecular principles of biofilm formation and dispersion**

**Molecular basis of dispersion and signal transduction**

K. Sauer<sup>1</sup>

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The process of biofilm dispersion represents an important phenotypic switch that allows cells residing within a biofilm to respond to changing conditions outside and within biofilm structures and to evade environmental stresses. Dispersion occurs in response to a wide array of signals, with dispersion-inducing conditions ranging from environmental cues to self-synthesized signaling molecules. Previous findings indicate biofilm dispersion by *Pseudomonas aeruginosa* to occur in response to various cues, to require BdlA and phosphodiesterases (PDEs, for instance DipA and RbdA), and to coincide with increased PDE activity and a reduction of c-di-GMP levels. However, little is known about dispersion cue sensing, the signaling events translating these cues into the modulation c-di-GMP levels, and the subsequent signaling cascade to enable dispersion resulting in the phenotypic switch. The presentation will focus on recent findings indicating how dispersion cue perception is translated across cellular compartments into the modulation of the intracellular c-di-GMP pool, the molecular events associated with signal transduction, and subsequent events associated with the induction of dispersion.

**FEMS-2675**

**Molecular principles of biofilm formation and dispersion**

**ADHESIN PROTEINS IN BACILLUS CEREUS BIOFILMS.**

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<sup>1</sup>Microbiología, Facultad de Ciencias., Málaga, Spain

**Background**

The human pathogen *Bacillus cereus* is responsible for many recurrent outbreaks of food poisoning. Spores and biofilms are considered the most important reservoirs of *B. cereus* in contaminated fresh vegetables and fruits. Bacterial biofilms are difficult to eradicate specially due to the presence of a protective extracellular matrix made of exopolysaccharides, proteins, and other components. Amyloid-like proteins are essential for the integrity of biofilms of the related bacteria species *Bacillus subtilis*.

**Objectives**

To investigate the presence of amyloid-like fibers in biofilms of *B. cereus*.

**Methods**

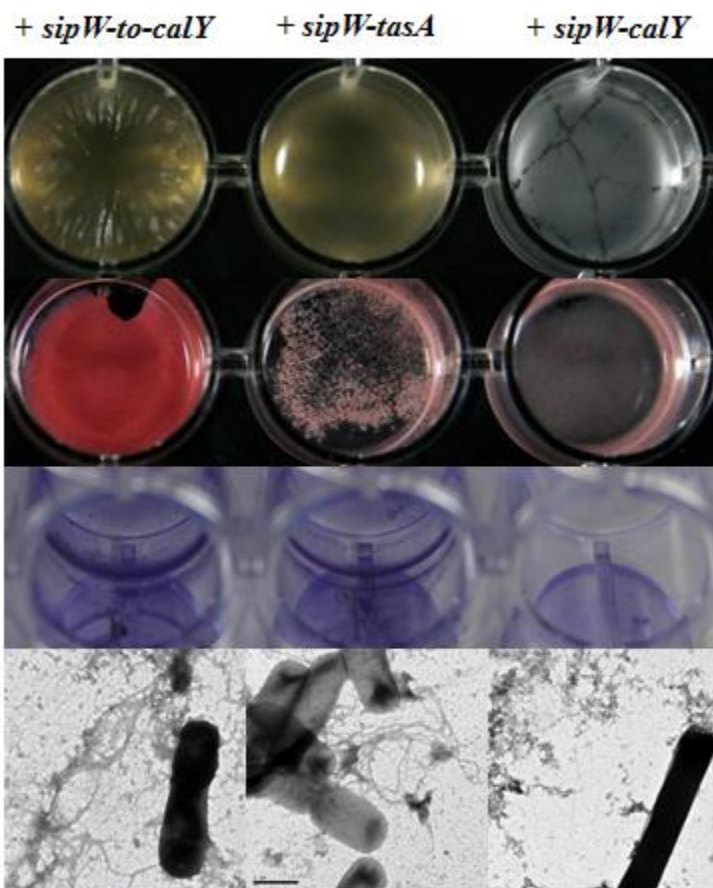
We identified two genomic loci in *B. cereus*, which encode two orthologues of the amyloid-like protein TasA and a SipW signal peptidase of *B. subtilis*. Mutagenesis in *B. cereus* or heterologous expression of alleles in *B. subtilis* mutants combined with crystal violet staining served to evaluate the formation of biofilm. Electron microscopy let us visualize the presence of fibers on cells.

**Conclusions**

We demonstrate that the proteins TasA and CalY are necessary for *B. cereus* biofilm formation: i) as pellicle on the air-liquid interphase or ii) adhesion to abiotic surfaces. TasA and to a lesser extent CalY polymerizes in the form of fibers in the cell surface. Our findings of heterologous expression in *B. subtilis* let us propose an amyloid-like

nature of the *B. cereus* TasA-based fibers.

*B. subtilis*  $\Delta$ operon



**FEMS-1178**

**Molecular principles of biofilm formation and dispersion**

**PSEUDOMONAS AERUGINOSA VIRULENCE FACTOR PYOCYANIN PROMOTES EXTRACELLULAR DNA RELEASE, INTERCALATES WITH DNA, CHANGES DNA PROPERTY AND ENHANCES BIOFILM FORMATION**

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<sup>1</sup>Department of Infectious diseases, University of Sydney, Sydney, Australia

<sup>2</sup>Department of Biotechnology and Biomolecular sciences, University of New South Wales, Sydney, Australia

<sup>3</sup>School of Chemistry, University of New South Wales, Sydney, Australia

**Background**

Approximately 80% of Cystic Fibrosis (CF) patients are infected with *P. aeruginosa*. *P. aeruginosa* infected lungs/sputum of CF patients contain pyocyanin up to 27.3 µg/ml. Pyocyanin is a potent virulence factor causing cell death in chronic lung infection in CF patients, thus increasing mortality. Studies suggest that pyocyanin influence *P. aeruginosa* biofilm formation. However, no work was done investigating the mechanism or functions of pyocyanin in biofilm formation.

**Objectives**

To elucidate the mechanism and functions of pyocyanin in *P. aeruginosa* biofilm formation and identify a novel therapeutic target to modulate pyocyanin functions thereby control *P. aeruginosa* biofilm formation.

**Methods**

eDNA concentration in *P. aeruginosa* culture was measured using Qubit 2.0 flurometer. Pyocyanin-eDNA binding mechanisms were analysed using spectrometer techniques. Influence of pyocyanin-eDNA interaction on viscosity of DNA solution and cell surface of *P. aeruginosa* strains were analysed using viscometer and surface thermodynamics approach respectively. Biofilm imaging and analysis of biofilm properties were performed using confocal microscopy. Antioxidants interaction with pyocyanin and inhibition of pyocyanin-eDNA binding were investigated using NMR and spectrometer techniques respectively.

**Conclusions**

Pyocyanin promotes eDNA release in *P. aeruginosa* wild-type by inducing cell lysis mediated through generation of H<sub>2</sub>O<sub>2</sub>. Pyocyanin intercalates into the nitrogenous bases of DNA and consequently increases DNA viscosity and influences *P. aeruginosa* cell surface hydrophobicity, physico-chemical interactions promoting bacterial interaction and biofilm formation. Pyocyanin deficient strain showed significant reduction in eDNA release and biofilm formation. Ascorbic acid and glutathione modulate pyocyanin functions thus inhibit pyocyanin-eDNA binding and consequently hamper biofilm development.

## FEMS-2506

### Molecular principles of biofilm formation and dispersion

#### BISTABLE GENE EXPRESSION IN SALMONELLA CONNECTS VIRULENCE TO PERSISTENCE

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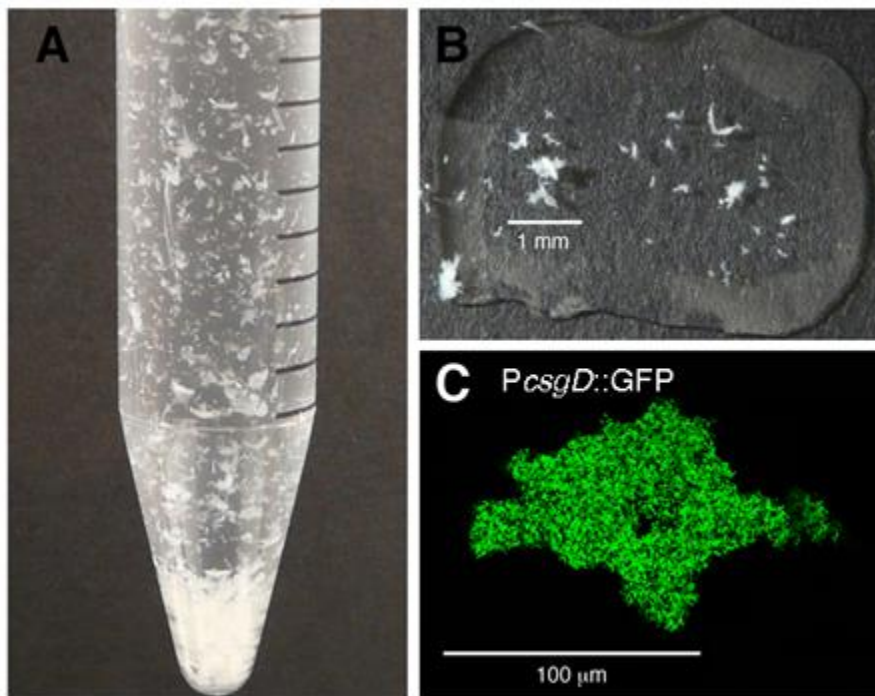
<sup>1</sup>Vaccine and Infectious Disease Organization, University of Saskatchewan, Saskatoon, Canada

#### Background

For the human and animal pathogen *Salmonella* Typhimurium, biofilm formation is correlated with persistence outside a host but the connection to virulence is unknown.

#### Objectives

To analyze specialized cells of *S. Typhimurium* that arise from bistable expression of CsgD, the central biofilm regulator, during growth under biofilm-inducing conditions.

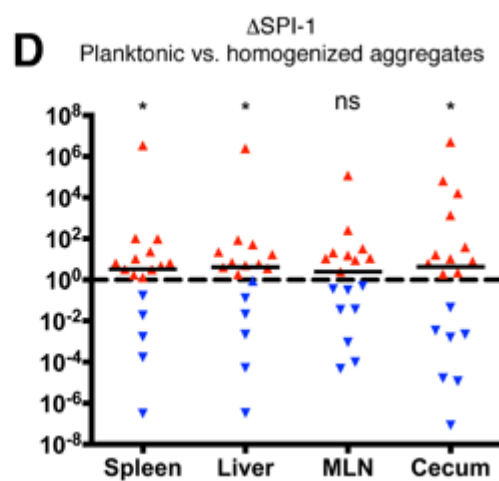
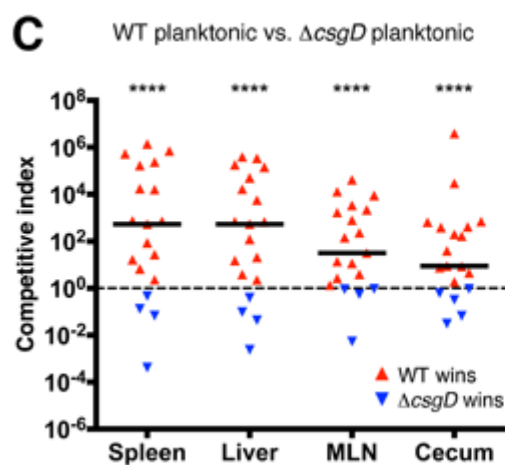
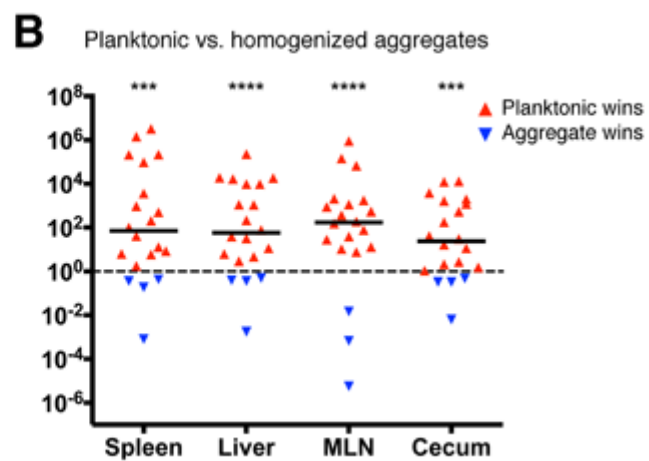
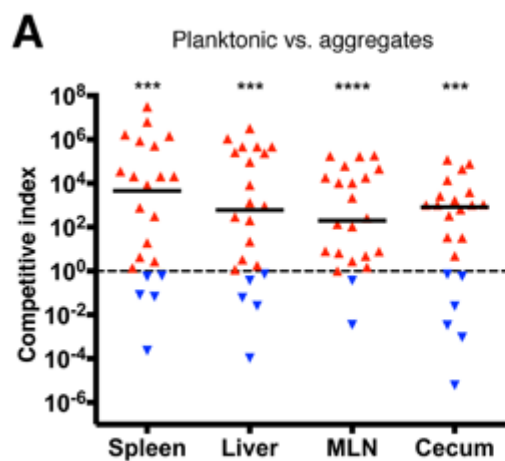


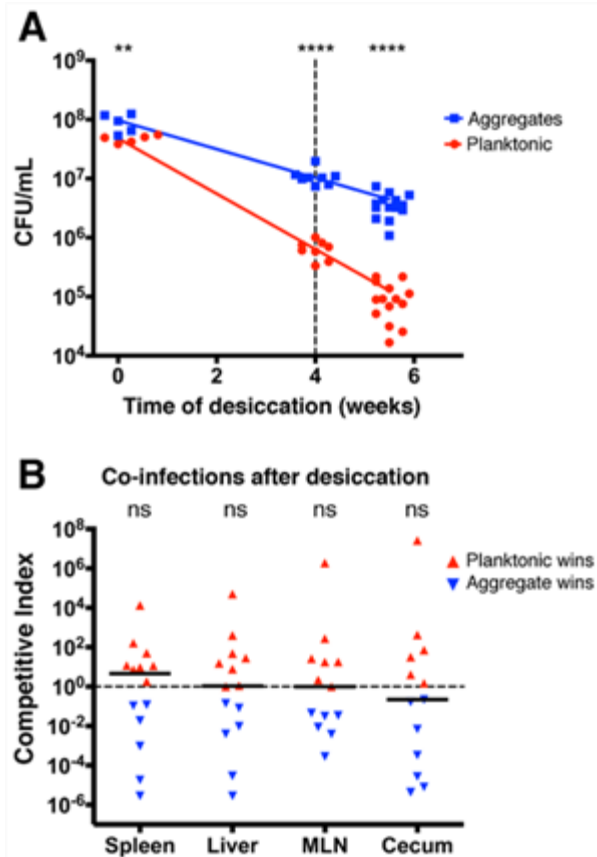
#### Methods

RNA-seq analysis of multicellular aggregates and planktonic cells identified 1856 genes, representing 34% of the genome, that were differentially expressed. Aggregated cells displayed the characteristic gene expression of biofilms, whereas

planktonic cells had enhanced expression of virulence genes, which were previously thought to be activated only by host conditions. Increased synthesis of the *Salmonella* pathogenicity island-1 type three secretion system in planktonic cells correlated with enhanced invasion of a human intestinal cell line and significantly increased virulence in mice as compared to the aggregates. However, when these same groups of cells were exposed to desiccation, the aggregates survived better and the competitive advantage of planktonic cells was lost.







## Conclusions

We hypothesize that CsgD-based differentiation is a form of bet-hedging for *S. Typhimurium*, with single cells primed for host cell invasion and aggregated cells adapted for persistence in the environment. This could be a common strategy for pathogens to spread the risks of transmission and ensure a smooth transition between the host and the environment.

**FEMS-2654**

## **Molecular principles of biofilm formation and dispersion**

### **GIL, A NEW C-DI-GMP-BINDING PROTEIN DOMAIN INVOLVED IN REGULATION OF CELLULOSE SYNTHESIS IN ENTEROBACTERIA**

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U. Römling<sup>2</sup>, M. Gomelsky<sup>1</sup>

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<sup>3</sup>National Center for Biotechnology Information National Library of Medicine, National Institutes of Health, Bethesda, USA

### **Background**

Numerous enzymes are involved in c-di-GMP synthesis and degradation in enterobacteria. In contrast, only a handful of c-di-GMP receptors/effectors have been identified.

### **Objectives**

The aim of this study was to identify new c-di-GMP receptors and to further characterize them.

### **Methods**

In search of new c-di-GMP receptors, we screened the *Escherichia coli* ASKA overexpression gene library using the Differential Radial Capillary Action of Ligand Assay (DRaCALA) with fluorescently and radioisotope-labelled c-di-GMP. We uncovered three new candidate c-di-GMP receptors in *E. coli* and characterized one of them, BcsE.

### **Conclusions**

The *bcsE* gene is encoded in cellulose synthase operons in representatives of Gammaproteobacteria and Betaproteobacteria. The purified BcsE proteins from *E. coli*, *Salmonella enterica* and *Klebsiella pneumoniae* bind c-di-GMP via the domain of unknown function, DUF2819, which is hereby designated GIL, GGDEF I-site like domain. The RxGD motif of the GIL domain is required for c-di-GMP binding, similar to the c-di-GMP-binding I-site of the diguanylate cyclase GGDEF domain. Thus, GIL is the second protein domain, after PilZ, dedicated to c-di-GMP-binding.

We show that in *S. enterica*, BcsE is not essential for cellulose synthesis but is required for maximal cellulose production, and that c-di-GMP binding is critical for BcsE function. It appears that cellulose production in enterobacteria is controlled by a two-tiered c-di-GMP-dependent system involving BcsE and the PilZ domain containing glycosyltransferase BcsA.

Fang et al., Mol Microbiol. 2014 Aug;93(3):439-52. doi: 10.1111/mmi.12672

**FEMS-0777**

**Host manipulation and bacterial survival Offered by Pathogens and Disease**

**Salmonella's intracellular toolkit**

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Following entry of *Salmonella* into host cells, this pathogen resides in a membrane-bound compartment called the *Salmonella*-containing vacuole (SCV). Here, the bacteria sense the acidic pH and poor nutritional status of the vacuole lumen and either enter a viable non-replicating state or begin to divide. Replication is dependent on activation of the SPI-2 type III secretion system (T3SS). The T3SS comprises an envelope-spanning secretion system and associated translocon pore in the vacuolar membrane. Bacteria then sense the near-neutral pH of the host cell cytoplasm; this results in dissociation and degradation of a bacterial membrane-bound regulatory complex, which activates translocation of approximately 30 bacterial effector proteins into the host cell.

We are currently studying the biochemical and physiological functions of various effectors, which have been implicated in several activities, including lysosomal detoxification, SCV localisation and membrane dynamics, interference with immune signalling and the induction of cytotoxicity. In this talk I will discuss our recent progress in these areas.

**FEMS-2853**

**Host manipulation and bacterial survival Offered by Pathogens and Disease**

**Manipulation of host membrane transport by Type IV effector proteins**

C. Roy<sup>1</sup>

<sup>1</sup>*Microbial Pathogenesis, Yale University School of Medicine, New Haven, USA*

Effector proteins delivered into the cytosol of host cells by Dot/Icm type IV secretion systems modulate processes important for creating a vacuole that supports bacterial intracellular replication. Here we describe the biochemical function of *Legionella pneumophila* and *Coxiella burnetii* effector proteins that play specific roles in controlling host membrane transport. These studies reveal new enzymatic activities and protein structures that demonstrate these pathogens encode novel effector proteins that can manipulate evolutionarily conserved host proteins that control membrane transport processes, which provide insight into how bacterial pathogens are able to construct a unique vacuole inside phagocytic host cells.

**FEMS-1844**

**Host manipulation and bacterial survival Offered by Pathogens and Disease**

**IDENTIFICATION OF LEGIONELLA PNEUMOPHILA VIRULENCE FACTORS  
REQUIRED TO SUBVERT HOST AUTOPHAGY**

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*International Center for Infectiology Research INSERM U1111 CNRS UMR 5308 EN  
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<sup>2</sup>*Autophagy Infections Immunity,*

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<sup>3</sup>*Evolutionary genetic and genomic,*

*Biometry and Evolutionary Biology UMR CNRS 5558, Villeurbanne, France*

**Background**

*Legionella pneumophila* is the causative agent of legionnaire's disease, a public health problem leading to at least 10% mortality rate. Via effectors secreted by its type IV secretion system, this intravacuolar bacterium interacts with many intracellular pathways of host cells including autophagy. This highly conserved pathway allows eukaryotic cells to recycle end-life cytosolic components in order to regulate cell homeostasis. Autophagy is also a degradative pathway essential to fight intracellular pathogen infections, but numerous microorganisms have evolved strategies in order to subvert this mechanism. The interaction between *L. pneumophila* and autophagy has been reported but remains still unclear.

**Objectives**

Our objective is to better decipher the *L. pneumophila*/autophagy interplay to determine whether this pathogen is controlled by autophagy or whether it gets benefit from this cellular process to survive/proliferate.

**Methods**

We used HeLa cells as a model to follow autophagy modulation upon *L. pneumophila* infection. Cells were infected with the northern Europe endemic strain *L. pneumophila* Paris.

**Conclusions**

Our experiments show that *L. pneumophila* infection induces autophagy in a type IV secretion system dependent manner, early post infection within infected cells but also in neighboring non-infected cells. Otherwise, preliminary data suggest that inhibition of autophagy is linked to a decreased bacterial replication, therefore autophagy could be beneficial for *L. pneumophila*. Thus, the autophagy stimulation could allow the acquirement of nutrients by the bacterium, provide membrane source for the expanding of *L. pneumophila* vacuole and/or lead to the delay of the fusion between *L. pneumophila* vacuole and lysosomes.





**FEMS-1274**

**Host manipulation and bacterial survival Offered by Pathogens and Disease**

**PASTEURELLA MULTOCIDA TOXIN MANIPULATES T CELL DIFFERENTIATION**

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<sup>1</sup>Department of Infectious Diseases, Medical Microbiology and Hygiene, Heidelberg, Germany

**Background**

*Pasteurella multocida* are gram-negative bacilli, which cause respiratory diseases in a number of wild and domestic animals. Toxigenic serotype A and D strains produce the *Pasteurella multocida* toxin (PMT), a classical AB toxin containing a deamidase activity in its catalytical domain. The toxin constitutively activates heterotrimeric G proteins and induces downstream signalling cascades involved in cytoskeleton rearrangement, proliferation, differentiation or survival of the host cell. Pathologically, PMT causes porcine atrophic rhinitis characterized by an increased number of osteoclasts and bone resorption at the nasal turbinate bones. However, PMT does not only act on cells of the bone system but we could demonstrate that it also modulates the signalling of many other hematopoietic cells such as B cells, macrophages or monocytes.

**Objectives**

We investigated how PMT treatment affects the differentiation of primary human T lymphocytes.

**Methods**

Proliferation studies showed that the toxin amplifies CD3/28 activated proliferation of T cells through the induction of cell cycle progression. Characterisation of transcription factor activation and the release of cytokines showed that PMT manipulates lineage determination towards T<sub>H</sub>17 and T<sub>reg</sub> cells as PMT-treated T cells are double positive for the lineage specific transcription factors FOXP3 (T<sub>reg</sub>) and RORγT (T<sub>H</sub>17) but predominantly behave like inflammatory IL-17-releasing T<sub>H</sub>17 cells.

**Conclusions**

These results indicate that a bacterial toxin is able to manipulate the direction of T<sub>H</sub>-cell differentiation and thereby presumably adjusts the sensitive balance of the immune response towards a condition that benefits the pathogen.



**FEMS-0663**

**Host manipulation and bacterial survival Offered by Pathogens and Disease**

**R-SPONDIN-2 MEDIATES SUSCEPTIBILITY TO INTESTINAL INFECTION WITH CITROBACTER RODENTIUM**

S. Gruenheid<sup>1</sup>, O. Papapietro<sup>1</sup>, S. Teatero<sup>1</sup>, A. Thanabalasuriar<sup>1</sup>, K. Yuk<sup>2</sup>, E. Diez<sup>1</sup>, L. Zhu<sup>1</sup>, E. Kang<sup>1</sup>, Y. Durocher<sup>3</sup>, M. Marcinkiewicz<sup>4</sup>, D. Malo<sup>2</sup>

<sup>1</sup>Microbiology and Immunology, McGill University, Montreal, Canada

<sup>2</sup>Human Genetics, McGill University, Montreal, Canada

<sup>3</sup>Biotechnology Research Institute, National Research Council of Canada, Montreal, Canada

<sup>4</sup>Cytochem, Cytochem Inc, Montreal, Canada

**Background**

*Citrobacter rodentium* is a mouse pathogen widely used as a model for Enteropathogenic and Enterohemorrhagic *Escherichia coli* infections in humans. While *C. rodentium* causes self-limiting colitis in most inbred mouse strains, it induces fatal diarrhea in hyper-susceptible strains.

**Objectives**

Here we sought to identify the gene and molecular mechanisms underlying this differential response to intestinal infection.

**Methods**

We used a forward genetics approach to unambiguously localize the locus conferring this differential outcome to a 4 Mb interval. Systematic evaluation of the genes within this region pinpointed *R-spondin -2* as the gene underlying infection susceptibility.

**Conclusions**

Robust induction of *R-spondin -2* expression during infection in susceptible mouse strains leads to pathological activation of WNT signaling, subsequent loss of intestinal differentiation, and animal death. Conversely, mouse strains that do not induce *R-spondin -2* expression following infection undergo milder, self-limiting disease with no mortality. Our data demonstrate a previously unknown role of R-spondins and WNT signaling in susceptibility to infectious diarrhea and identify *R-spondin -2* as a key molecular link between enteric infection and control of intestinal homeostasis.

**FEMS-2272**

**Host manipulation and bacterial survival Offered by Pathogens and Disease**

## **THE ROLE OF GLYCOGEN DEGRADATION AND METABOLISM IN BACTERIAL VAGINOSIS**

R. Hertzberger<sup>1</sup>, A. Lewis<sup>1</sup>, W. Lewis<sup>1</sup>

<sup>1</sup>*School of Medicine, Washington University, St. Louis, USA*

### **Background**

Bacterial vaginosis (BV) is a common condition of the human vagina in which the lactobacilli that normally dominate this niche are absent and replaced by a plethora of fastidious anaerobes. BV is associated with an increased risk of acquiring sexually transmitted disease and adverse health outcomes during pregnancy. Factors that drive the microbial dynamics in BV are poorly defined.

Glycogen is deposited in high levels in human vaginal epithelial cells. It has been postulated to be a major carbon source for vaginal bacteria.

### **Objectives**

Here we test the hypothesis that BV-associated bacteria digest and metabolize glycogen.

### **Methods**

We evaluated glycogen degrading enzyme activity in vaginal specimens of women with and without BV. Growth assays were conducted to examine the ability of lactobacilli and BV-associated bacteria to utilize glycogen. We examined evidence for glycogen-degrading enzymes among vaginal bacteria.

### **Conclusions**

These experiments provide strong evidence that BV associated bacteria degrade glycogen and metabolize the released sugar residues, whereas most vaginal lactobacilli do not utilize glycogen. These results provide an explanation of the recent observation by Mirmonsef et al. (1) that glycogen levels in BV are reduced.

1. Mirmonsef P, Hotton AL, Gilbert D, Burgad D, Landay A, Weber KM, Cohen M, Ravel J, Spear GT. 2014. Free glycogen in vaginal fluids is associated with Lactobacillus colonization and low vaginal pH. PLoS One. 9:e102467.



**FEMS-2874**

**Fungal plant pathogens**

**Transcriptional networks controlling pathogenicity and polarized growth in *U. maydis***

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<sup>1</sup>*Institute for Applied Biosciences, Karlsruhe Institute of Technology, Karlsruhe, Germany*

<sup>2</sup>*Institute for Microbiology and Genetics, University of Göttingen, Göttingen, Germany*

The transition from yeast-like saprophytic growth to the formation of pathogenic filamentous hyphae is a critical step in the life cycle of the basidiomycete *Ustilago maydis*. The switch is controlled by a pheromone/receptor system and the heterodimeric bE/bW transcription factor complex, encoded by the *a* and *b*-mating type loci, respectively. As both pathways control polar growth and cell cycle, they have to be closely interconnected and cross-controlled. The transcription factor Rbf1, a central node for gene regulation during pathogenic development, responds to inputs from both *a* and *b* pathways. Coordination is achieved via the interaction of Rbf1 and bW with the Clp1 protein that renders the bE/bW complex inactive and converts Rbf1 to a repressor of the pheromone pathway, releasing both the *a* and bE/bW mediated cell cycle arrest. We are currently investigating the molecular mechanisms by which the factors independently, cooperatively, and after interaction with Clp1, regulate gene expression during the dimorphic switch, using a combination of reporter gene assays, *in vitro* DNA binding studies and the genome-wide identification and occupation of promoter binding sites.

$\Delta clp1$  strains are capable to infect maize plants, but, as the cell cycle block is not released, development is stalled after formation of the appressorium, before the first cell division. In both wildtype and  $\Delta clp1$  hyphae, nuclear envelopes start to dissolve before appressorium formation, which is typical for nuclei entering mitosis. In wildtype cells, nuclei migrate into the invading hyphae and divide. However, nuclei of  $\Delta clp1$  cells remain in the appressorium, suggesting a novel function for Clp1 in the coordination of nuclear division and nuclear migration.

**FEMS-2833**

**Fungal plant pathogens**

**Pathogenomics of *Verticillium* wilt diseases**

B. Thomma<sup>1</sup>, M. Seidl<sup>1</sup>, X. Shi<sup>1</sup>, J. Boshoven<sup>1</sup>, M. van Damme<sup>1</sup>, J. Li<sup>1</sup>,  
E. Rojas Padilla<sup>1</sup>, Y. Song<sup>1</sup>, G. van den Berg<sup>1</sup>, L. Faino<sup>1</sup>

<sup>1</sup>Laboratory of Phytopathology, Wageningen University, Wageningen, Netherlands

Fungi cause severe crop losses and threaten food security worldwide. The soil-borne fungal pathogen *Verticillium dahliae* causes vascular wilt disease on hundreds of plant species, and disease control is challenging because resistance in plants is relatively rare. Moreover, *V. dahliae* has a flexible genome allowing it to escape host immunity and maintain aggressiveness. So far, knowledge on mechanisms governing this genomic flexibility remains limited.

Through comparative population genomics we have started to unravel mechanisms to establish the genomic diversity that is essential for adaptive genome co-evolution during the continued arms race with host plants. To this end, two *V. dahliae* genomes were assembled from telomere-to-telomere using long-read sequencing technology and optical mapping, and compared these to the genomes of other *Verticillium* spp., revealing a pre-speciation genome duplication event. Comparative genomics using the two finished *V. dahliae* genomes furthermore revealed recent segmental duplications that established lineage-specific regions. Interestingly, these regions are enriched for in planta-expressed effector genes encoding secreted proteins that enable host colonization, and thus contribute to the evolution of virulence. Our evidence suggests that error-prone homology-dependent DNA repair has caused genomic rearrangements, leading to extensive structural variations. Re-sequencing of additional strains showed that independent losses of genetic material favored the escape of host recognition and, likely, host specificity. We propose that evolution of *V. dahliae* is linked to segmental genome duplications mediated by improperly repaired DNA breaks.

In addition to genome evolution, we also study the role of epigenetic modifications on virulence of *V. dahliae* and the biological functions of effector proteins. Collectively, these research lines provide insight in mechanisms that make this fungus such a successful broad host range pathogen.

**FEMS-1897**

**Fungal plant pathogens**

**FGSSP6, A CERATO-PLATANIN PROTEIN WHICH CONTRIBUTES TO FUSARIUM GRAMINEARUM VIRULENCE ON WHEAT SPIKES.**

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**Background**

*Fusarium graminearum* (*Fg*) is one of the main fungal pathogens of wheat crops globally, causing Fusarium ear blight (FEB) disease. Like many other plant pathogens, *Fg* is predicted to produce *in planta* various secreted effector proteins that modulate plant metabolism to suppress or re-programme plant defences. These induced changes enable successful colonisation of plant tissues.

**Objectives**

To identify *Fg* effector proteins that can suppress host plant defences

**Methods**

We selected a set of small secreted proteins (SSP) to express *in planta* using the *Barley stripe mosaic virus* over-expression system (BSMV-VOX) and tested whether any enhance *Fg* fungal infection of susceptible wheat spikes.

**Conclusions**

Amongst the initial set of *Fg* SSP tested, one called FgSSP6, which belongs to the cerato-platanin protein (CPP) family, caused the quicker development of FEB disease symptoms in wheat ears following point inoculation. Bleaching of all spikelets on wheat spikes occurred by 12 days post inoculation (dpi) in the presence of BSMV:FgSSP6, whereas with various BSMV control vectors, full *Fg* infection took 16 dpi or longer. In several other plant pathogenic fungi, CPPs are already known to act as key virulence factors. So far our results suggest that FgSSP6 does not induce necrosis to assist infection, but either suppresses plant defence responses or plays a novel virulence role. This is the first report of the successful use of the BSMV-VOX system in combination with the *Fg*-wheat spike infections to identify novel



candidate *Fg* effectors. Further studies are planned to clarify the precise *in planta* role(s) of this newly identified cerato-platanin effector.

**FEMS-0840**

**Fungal plant pathogens**

**A NOVEL METHOD FOR RAPIDLY ISOLATING FUNGAL SUPPRESSIVE MICROBES DIRECTLY FROM SOIL**

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**Background**

Soilborne fungal root phytopathogens are some of the most damaging and difficult to control pathogens faced by agricultural production in the field, nurseries and greenhouses worldwide. There are many detrimental issues with current chemical control methods and this has increased interest in biological control using soil microorganisms that suppress the growth of phytopathogens. Antibiosis is one of the most important mechanisms responsible for fungal antagonism, with some significant antifungal compounds including, antibiotics, volatile organic compounds, hydrogen cyanide and lytic enzymes (Bhattacharyya & Jha, 2012; Compant *et al.*, 2005). Up to date methods for the isolation of fungal-suppressive microorganisms from the soil are time consuming and tedious.

**Objectives**

The aim of this study was to establish a simple method for isolating fungal pathogen-suppressive microbes (bacteria and fungi) directly from the soil as well as procedures for confirmation of disease suppression.

**Methods**

We will report on these methods, which were so far tested with three cotton fungal pathogens *Thielaviopsis basicola*, *Verticillium dahliae* and *Fusarium oxysporum* and a pathogen of button mushrooms *Verticillium fungicola*.

**Conclusions**

We have isolated a diversity of *T. basicola*- suppressive fungi and bacteria from two vastly different soil types. Identification of the antagonistic isolates revealed that they are a diverse lot, some belonging to groups known to be suppressive of a wide range of fungal pathogens, endorsing the power of this technique to rapidly and directly isolate soil-borne microbes antagonistic to a wide variety of fungal pathogens.

**FEMS-0852**

**Fungal plant pathogens**

**EVALUATION OF GENETIC DIVERSITY AMONG PHYTOPATHOGENIC ISOLATES OF FUSARIUM SOLANI COMPLEX CAUSING SHISHAM DIEBACK DISEASE IN PAKISTAN**

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**Background**

*Dalbergia sissoo* Roxburgi, (shisham) is an important and established forest species in south Asia. However, this precious tree is facing many devastating challenges from last six decades that dragging it towards disappearance from the subcontinent. Dieback is one of main diseases in Shisham plantations and caused by *Fusarium solani*.

**Objectives**

The aim of this study was to investigate the regional dependant genetic variation among different pathogenic *F. solani* isolates and a link to persistence cause of shisham dieback in various agro ecological zones.

**Methods**

A total of 23 pathogenic representative isolates of *F. solani*, collected from various agro ecological zones were subjected to analysis of genetic variability in terms of DNA polymorphism using RAPD-PCR. Results were obtained with the help of 10 randomly amplified polymorphic DNA markers (OPA1-OPA10) to test genetic variability in *F. solani* isolates.

**Conclusions**

A total of 238 amplified products generated with the primers and 23 isolates of *F. solani*. The pattern of genetic variability in the isolate was also supported by the analysis of the similarity indices and UPGMA dendrogram. Twenty three isolates of *F. solani* recovered from different areas showed a significant genetic variation during RAPD analysis. It seems that the genetic variability among fungal isolates is regional dependent and reason of persistent cause of shisham dieback in Punjab, Pakistan. Current findings showed the variation among pathogenic isolates of *F. solani* and

revealed that persistent cause of shisham dieback is due to *F. solani* complex in different shisham growing regions in province Punjab, Pakistan.

## **FEMS-1202**

### **Fungal plant pathogens**

#### **APPLICATION OF TRICHODERMA STRAINS AGAINST GTD PATHOGENS**

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#### **Background**

The Grapevine Trunk Diseases are one of the most prevalent diseases of the vineyard worldwide, caused by a variety of fungal pathogens. Mycoparasites, like *Trichoderma* species are potential biopesticides against GTD pathogens.

#### **Objectives**

The aims of this study were taxonomical identification of ten *Trichoderma* sps. from asymptomatic plants in the Tokaj wine region the determination of mycelial growth rate at different temperatures, the mycoparasitic potential against *Diplodia seriata* and *Neofusicoccum parvum* and field experiments with the mixed *Trichoderma* spore suspension.

#### **Methods**

The *Trichoderma* isolates were identified based on their ITS1,2 and *tef1* marker sequences. Their mycelial growth was determined from the two average colony diameters on PDA, measured for each *Trichoderma* isolates for 4 days. The mycoparasitic ability was detected according the method of Szerekes et al (2006; *Journal of Microbiological Methods*, 619-622). Some GTD symptomatic plants were treated with *Trichoderma* spore suspension in 2014. The treated trunks were checked monthly.

#### **Conclusions**

The ten isolates belonged to three species: *T. harzianum* (TR01-05, TR07, TR09-10), *T. orientalis* (TR06) and *T. viride* (TR08). The *Trichoderma* isolates showed different mycelial growth rate on different temperatures (5 - 37°C). The *T. orientalis* (TR06) and *T. harzianum* (TR05) isolates showed the highest growth rates within the whole temperature range, and their growth rates were especially high at 30 and 37°C. All *Trichoderma* overgrew the GTD pathogens, and sporulated on their colonies, therefore their Biocontrol Index was 100%.

The symptoms decreased on all treated samples, except one. Moreover the *Trichoderma* strains could be reisolated from their woody tissues.

**FEMS-2811**

**Genomics, evolution, phylogeny**

**DNA substrates for horizontal gene transfer by natural transformation**

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The dynamic composition of the genomes of free-living bacteria challenges our understanding of microbial species. Horizontal gene transfer contributes to this dynamics and enables the ongoing dissemination of antimicrobial resistance genes across bacterial populations. Thus, there is a immediate need for a better understanding microbial genome dynamics both on a short-term ecological and long-term evolutionary scale. Natural transformation is a gene transfer mechanism that can both lead to intra-genomic rearrangement and inter-genomic DNA recombination. Here I present some recently experimentally derived results that advance our understanding of how species-foreign DNA fragments can be acquired by the naturally transformable model bacterium *Acinetobacter baylyi*, and describe the fate of such DNA after initial recombination with the host genome. Extracellular DNA in decaying organisms becomes chemically modified and degraded over time. However, it is known that from paleogenetics that short DNA fragments remain over time in some environments. The newly discovered opportunity for bacteria to acquire very short DNA fragments suggest extinct genomes can be an additional source of DNA for naturally transformable organisms.

**FEMS-2859**

**Genomics, evolution, phylogeny**

**Evolution of a superbug: Real-time transfer of antimicrobial resistance in the host**

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***Staphylococcus aureus* is a commensal and major pathogen of humans and animals, capable of carrying a wide range of mobile genetic elements (MGEs) encoding antimicrobial resistance (AMR), virulence and host adaptation pathways. Whole genome analysis reveals a population structure consisting of independently evolving lineages, each defined by variation in core genomes. Within lineages, MGEs vary substantially with potential implications for selection in various hosts and under antimicrobial pressures. Little is known about the process of MGE transfer *in vivo*, nor how *S. aureus* adapt to antimicrobials or new hosts in real time. We co-colonized gnotobiotic piglets with both human- and pig-associated variants of the lineage clonal complex (CC)398, and observed transfer of MGEs including resistance genes from the pig to the human isolate within 4 hours. Extensive and repeated transfer resulted in colonization with isolates carrying a wide variety of MGE combinations. Whole genome sequencing of progeny bacteria revealed no acquisition of core genome polymorphisms. Separate studies of human hospitalized patients colonized with methicillin-resistant *S. aureus* (MRSA) CC22 revealed clonal populations varying in antimicrobial resistance profiles and MGE carriage, as well as free bacteriophage capable of gene transfer by generalized transduction. Our data show that transfer of AMR can occur at very high frequency *in vivo* and at higher frequency than detected *in vitro*.**



**FEMS-1685**

**Genomics, evolution, phylogeny**

**PLASTICITY OF EVOLVABILITY DRIVES EVOLUTION TO HIGH ETHANOL TOLERANCE IN ESCHERICHIA COLI**

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**Background**

Ethanol tolerance is a complex trait requiring interaction between different genes and pathways. Exposure to ethanol is toxic and slows down the cellular growth rate. Consequently, evolving high ethanol tolerance is slow and depends on the simultaneous occurrence of different mutations. This is unlikely in wild-type cells with a low mutation rate. In contrast, the odds that different mutations occur simultaneously are higher in hypermutable strains. These mutators are known to have an increased evolvability on a short evolutionary timescale, but display decreased fitness as the genetic load progressively becomes more deleterious.

**Objectives**

We aim at understanding the evolutionary mechanisms underlying adaptation to ethanol. This can lead to new insights that are also relevant for other complex phenotypes. Additionally, we investigate the role of a mutator phenotype in evolution towards high ethanol tolerance. This can shed light on the way bacteria adapt to severe stress conditions.

**Methods**

Here we used experimental evolution to study mechanisms underlying evolution to high ethanol tolerance in *E. coli*. Furthermore, through fluctuation assays and competition assays we determined mutation rates and established the advantage or disadvantage of hypermutation at different times during adaptive evolution.

**Conclusions**

We show that an increased mutation rate is essential for *E. coli* to adapt to high ethanol levels under slow-growing conditions. In direct competition under ethanol stress hypermutators outcompete wildtype cells. Moreover, we demonstrate that the population mutation rate decreases again when high ethanol tolerance is reached, indicating that once a population reaches a fitness peak, it strives to preserve the genome.

**FEMS-1739**

**Genomics, evolution, phylogeny**

**REPLICATION-DEPENDENT GENE DOSAGE OF RIBOSOMAL PROTEINS  
REGULATES GROWTH RATE OF *VIBRIO CHOLERA*E.**

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**Background**

The effects of gene order within the bacterial chromosome on cell physiology are poorly understood. *In silico* approaches have showed that ribosomal protein genes locate near the replication origin (*oriC*) in fast-growing bacteria, suggesting that such a positional bias is an evolutionarily conserved growth-optimization strategy. *Vibrio cholerae*, a bi-chromosomal fast-growing pathogen is a well-characterized model organism to experimentally tackle this hypothesis. The *S10-spc-α* locus (S10) encodes half of the ribosomal protein genes and is conserved along all life forms.

**Objectives**

To relocate S10 to different positions along the genome and measure putative physiological effects.

**Methods**

We used novel recombineering tools to create a set of isogenic strains in which S10 was systematically relocated to alternative genomic positions. Their DNA and RNA was analyzed using NGS.

**Conclusions**

We show that S10 relative distance to the *oriC* tightly correlates with a reduction of its dosage, mRNA abundance and growth rate. This is accompanied by a significant reduction in host-invasion capacity of *Drosophila melanogaster*. Strains bearing two S10 copies far from *oriC* rescue both phenotypes demonstrating that replication-dependent gene-dosage reduction is the main mechanism behind these alterations. Deep sequencing analyses shows that i) the whole *oriC* region is less transcribed in the most affected derivatives and ii) Gene Set Enrichment Analyses reveal 'translation' as the most altered pathway in such derivatives. S10 positioning connects genome structure to cell physiology in *V. cholerae*. We show that genomic positioning of genes involved in the flux of genetic information conditions global growth and hence bacterial physiology and evolution.

**FEMS-2140**

**Genomics, evolution, phylogeny**

## **EVOLUTION AND PATHOADAPTATION OF PSEUDOMONAS AERUGINOSA IN CYSTIC FIBROSIS PATIENTS**

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### **Background**

Laboratory evolution experiments have led to important findings addressing to what extent natural selection constrains parallel evolving organisms to follow common evolutionary pathways towards adaptive phenotypes, and in which way evolutionary outcomes become intertwined over time such that future alternatives may be contingent on the prior history of an evolving population. Nonetheless, similar systematic investigations of bacterial populations living in complex natural environments are lacking, limiting our understanding of these processes in situ.

### **Objectives**

To address questions about the role of evolutionary convergence and historical contingency in a “natural evolution experiment”, we investigate the molecular evolution of distinct clonal lineages of *Pseudomonas aeruginosa* from the initial invasion into the cystic fibrosis (CF) airways and onwards as they genetically adapt to a human host after transition from their environmental habitat.

### **Methods**

We sequenced and analyzed the genomes of >500 longitudinally collected clinical isolates of *P. aeruginosa* sampled from >40 CF patients.

### **Conclusions**

Parallel evolution across multiple *P. aeruginosa* strains identified convergent molecular evolution in pathoadaptive genes that revealed host-adaptation to involve remodeling of regulatory networks and central metabolism, acquisition of antibiotic resistance, and loss of extracellular virulence factors. Furthermore, we demonstrated how historical contingency played a role in on the evolution of regulatory networks, in which mutations in downstream transcriptional regulators were contingent upon mutations in upstream regulators; suggesting that remodeling of regulatory networks facilitate adaptation. Knowledge of pathoadaptive mutations and evolutionary contingency may help prediction of bacterial evolution in CF patients and design of future intervention strategies.

**FEMS-2960**

**Genomics, evolution, phylogeny**

**Mäkelä-Cassell Awardee: Taxon-specific growth rates are related to phylogeny in soil bacterial communities**

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Understanding if and how the phylogenetic composition of microbial communities is related to ecosystem function has been a central question in microbial ecology. To date, attempts to unambiguously identify the microbial taxa involved in an ecosystem process of interest have relied on stable isotope probing (SIP), where heavy isotopes are incorporated into the nucleic acids of microorganisms utilizing a compound of interest (<sup>13</sup>C carbon sources) or actively growing (<sup>18</sup>O water). Traditionally, this method has been qualitative. However, we have advanced this technique in order to quantitatively assess the amount of isotope incorporation into nucleic acids of specific taxa. This is achieved by comparing the naïve density of a DNA fragment to its density after incubation in the presence of a stable isotope, as the change in density is proportional to the amount of stable isotope incorporation. Using this technique, we have estimated the taxon-specific growth rates of soil bacteria under control conditions and in the presence of an added substrate (<sup>13</sup>C-glucose). The growth rates and <sup>13</sup>C assimilation rates of the most abundant taxa were compared to their phylogenetic relatedness to determine if there was a phylogenetic signal (measured using Blomberg's K test) associated with the growth response. Indeed, closely related organisms were similar in their growth responses. This phylogenetic signal was found after single (K=0.81, p<0.001) and repeated substrate addition (K=0.99, p<0.001), as well as in the absence of added substrate (K=0.73, p<0.001). The different resource conditions elicited growth of distinct, phylogenetically-clustered groups suggesting a microorganism's taxonomic identity could be a useful indicator of its ecology in the environment. Generally, these data suggest that the growth responses and therefore nutrient transformations of soil microorganisms are significantly influenced by evolutionary history.

**FEMS-2840**

**Antibiotics: novel approaches to treat resistant organisms**

**Compounds for Overcoming Antibiotic Tolerance and Resistance**

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**Compounds for Overcoming Antibiotic Tolerance and Resistance**

Currently available antibiotics are largely inactive against dormant persister cells produced by microbial pathogens, and are prone to resistance development. Persisters are responsible for the chronic, relapsing nature of the disease. An even more daunting unsolved problem is the seemingly inevitable development of resistance to all known antibiotics. We find that different natural compounds targeting the Clp protease, acyldepsipeptide and lassomycin, kill persisters and eradicate the pathogen population. Teixobactin, a cell wall synthesis inhibitor was discovered in a screen of uncultured soil bacteria. Teixobactin is the first member of a new class of cell wall acting inhibitors and binds two targets, lipid II, precursor of peptidoglycan, and lipid III, precursor of wall teichoic acid. The targets are not proteins, and there is no resistance development to this compound. Soil microorganisms are likely to harbor many more sterilizing and largely resistance-free compounds. Screening uncultured bacteria provides an effective platform for antibiotic discovery.

**FEMS-1059**

**Antibiotics: novel approaches to treat resistant organisms**

**Exploring microbial natural products as sources for novel antibiotics**

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Infectious diseases are one of the leading causes of death worldwide and the emergence of multi-drug-resistant bacteria, and the lack of new antibiotics in development with new modes of action are widely accepted as one of the major clinical concerns that require to be addressed from the public and private sectors. One of major clinical needs are related with MDR Gram-negative bacteria hospital-acquired infections, frequently associated with high mortality rates and few treatment options. Very limited options have been developed to date against Gram negative, and even last resorts antibiotics such as colistin and polymixin B are facing resistances.

Microbial natural products (NPs) have been one of the most prolific sources of new leads for the discovery of novel antibiotics, with a large number of compounds and analogs successfully introduced in the market in the past decades and still today in the clinic. NPs potency and selectivity is the result of an extended evolutionary selection to create biologically active molecules with the required properties to penetrate bacterial membranes and interact and potentially inhibit bacterial targets.

The strategy developed atMEDINA for the discovery for novel antibiotics is focused on the exploitation of our proprietary NPs collections, one of the most diverse and continuously expanding sources of novel compounds. Most recent efforts have permitted the identification of novel families of compounds that will be discussed in the context of current antibiotic discovery efforts in the academia and the pharma sector.

**FEMS-2297**

**Antibiotics: novel approaches to treat resistant organisms**

**Xenorhabdus & Photorhabdus: a Promising Bioresource for the Discovery of Novel Antibiotics**

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Antibiotic resistance remains a high unmet medical need. The situation is worsened by the scarcity of investigational new drugs in the antibacterial pipeline, especially against multidrug-resistant Gram-negative pathogens (*Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*). Novel antibiotic classes are clearly needed to overcome the current mechanisms of resistance faced by the clinicians. Among the different approaches to discover novel antibacterial classes, the mining of the microbial biodiversity is one of the most promising. In this context, the bacterial genera *Xenorhabdus* and *Photorhabdus* represent a high-potential bioresource that remains to be extensively explored. *Xenorhabdus* and *Photorhabdus* are symbiotic bacteria of the entomopathogenic nematodes *Steinernema* and *Heterorhabditis*. They have an original life-cycle that requires the production of a great diversity of bioactive secondary metabolites, including antimicrobials. This ecological rationale is supported by a high content of PKS and NRPS genes in their genomes. The screening of a collection of *Xenorhabdus* and *Photorhabdus* strains led to the discovery of the Odilorhabdins, a novel antibiotic class with a broad spectrum activity against Gram-positive and Gram-negative pathogens, including multidrug-resistant clinical strains. The Odilorhabdins have shown efficacy in murine infection models. They are currently undergoing preclinical optimization by medicinal chemistry. Odilorhabdins are expected to enter into clinical trials in human by 2017.

**FEMS-2855**

**Antibiotics: novel approaches to treat resistant organisms**

**$\beta$ -Hairpin peptidomimetic antibiotics targeting outer membrane proteins in Gram-negative bacteria**

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The biogenesis of the asymmetric outer membrane (OM) of Gram-negative bacteria, with lipopolysaccharide in the outer leaflet and phospholipid in the inner leaflet, and adorned with lipoproteins and a large family of  $\beta$ -barrel OM proteins, is an attractive target for the development of new antibiotics. One approach focuses on naturally occurring cationic antimicrobial host defense peptides (HDPs), which form part of innate immunity in many organisms and provide a first line of protection against microbial infection. In the pharmaceutical sciences as a whole it is well known that when linear peptides are constrained into unique backbone conformations, peptidomimetics can sometimes be found with target specificity and affinity not seen with unconstrained peptides. As an example, I will describe in this lecture a family of constrained  $\beta$ -hairpin-shaped cationic antimicrobial peptidomimetics derived from a HDP, with potent and selective antimicrobial activity against *Pseudomonas* spp. These peptidomimetics were shown to target the  $\beta$ -barrel outer membrane protein LptD in *P. aeruginosa* and to inhibit its key function in LPS transport during outer membrane biogenesis. This represents a novel mechanism of action that has not been described so far in any naturally occurring HDP or antimicrobial natural product. A clinical candidate is now in phase-II clinical studies in patients with serious hospital-acquired lung infections.

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**FEMS-0606**

**Cyclic di-nucleotides in bacteria**

## **C-DI-GMP SIGNALING AND E. COLI BIOFILM ARCHITECTURE**

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### **Background**

Bacterial biofilms contain matrix-producing and matrix-free cells in distinct zones. In *E. coli* K-12 the production of matrix components such as amyloid curli fibres and cellulose is promoted by the second messenger c-di-GMP, which is synthesized by 12 diguanylate cyclases (DGC) and degraded by 13 phosphodiesterases (PDE). Two PDE/DGC pairs (YhjH/YegE and YciR/YdaM) constitute a bistable switch device that can sharply drive up the cellular c-di-GMP level in slowly growing and stationary phase zones of macrocolony biofilms and thereby turn on the expression of the biofilm regulator CsgD. This transcription factor then activates expression of the curli genes as well as of *yaiC*, which encodes a DGC specifically required for cellulose synthesis.

### **Objectives**

Recent progress in understanding this c-di-GMP signaling network will be presented.

### **Methods**

Genetics, biochemistry, microscopy, mathematical modelling.

### **Conclusions**

In the central c-di-GMP signaling network that controls *E. coli* biofilm matrix production, YegE is the 'starter' DGC, which - by producing c-di-GMP that is sensed by the 'trigger enzyme' YciR - promotes YdaM activity and YaiC expression. These two DGCs further increase c-di-GMP levels and thereby form a positive feedback loop. In addition, most of the other DGCs and PDEs are expressed but do not contribute to this scenario under standard conditions, suggesting that these enzymes are inactive. However, specific signals perceived by the various N-terminal sensor domains may activate these DGCs and PDEs and thereby modulate c-di-GMP levels, matrix production and biofilm morphology. As an example a novel subfamily of c-di-GMP-related enzymes will be presented whose activity is regulated by redox signal input.

**FEMS-0896**

**Cyclic di-nucleotides in bacteria**

**c-di-AMP signalling in *Staphylococcus aureus***

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Signalling nucleotides are key molecules in all domains of life and control fundamental processes including central metabolic or stress response processes as well as biofilm formation. c-di-AMP is one of the more recently discovered signalling molecules and is produced by a number of bacteria including pathogens such as *Staphylococcus aureus*. In *S. aureus*, c-di-AMP is synthesised from two molecules of ATP by the diadenylate cyclase DacA and degraded to pApA by the phosphodiesterase GdpP. c-di-AMP has since been implicated in controlling cell size in *S. aureus*, in helping this microorganism cope with cell wall stress and in the regulation of potassium uptake. Here, the identification and function of c-di-AMP receptor proteins will be discussed. Furthermore, the apo- and c-di-AMP complex structures of PstA, one of the c-di-AMP target proteins, will be presented. This allowed us to propose a mechanism on how PstA functions as a signaling transduction protein. The complex structure also highlighted common features of c-di-AMP binding sites allowing for a more rational prediction of cyclic-di-AMP binding sites.

## FEMS-1341

### Cyclic di-nucleotides in bacteria

#### **Chp8, a composite diguanylate cyclase which promotes *Pseudomonas syringae* pv tomato DC3000 pathogenicity and plant immune evasion during infection of *Arabidopsis thaliana***

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*Pseudomonas syringae* (*Ps*) is a bacterial pathogen which interacts with more than 50 different plants causing diseases in crops including tomato, beans and rice. The associated decrease in crop yields results not only in economic losses, but also threatens global food security. *Ps* uses type-three-secretion (TTSS) to deliver virulence factors into the plant that promote survival of the bacterium and ultimately results in disease symptoms. The *Ps* TTSS is a product of the *hypersensitive response and pathogenicity* (*hrp*) and *hypersensitive response and conserved* (*hrc*) gene cluster under the control of sigma<sup>54</sup>, the co-dependent transcriptional activators HrpRS and the alternative sigma factor HrpL.

Here, I will present our findings that in the model strain *Ps* DC3000 HrpRS also activate expression of the composite diguanylate cyclase Chp8 (Co-regulated with hrp 8) in response to plant derived signals. Chp8 diminishes the hormonal immune response of *Arabidopsis thaliana* and promotes *Ps* DC3000 pathogenicity. Chp8 is the first example of a diguanylate cyclase promoting pathogenicity of a bacterial plant pathogen. The study was recently published in *mBio* (Engl *et al.*, 2014) and appraised as the first complete description of how a diguanylate cyclase can affect bacterial-host interactions in an acute infection model.

**FEMS-0860**

**Cyclic di-nucleotides in bacteria**

**THE ROLE OF CYCLIC DI-GMP IN STREPTOMYCES DEVELOPMENT**

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**Background**

*Streptomyces* are non-motile soil bacteria with a unique mycelial, sporulating life cycle and the ability to produce highly valuable secondary metabolites. *Streptomyces venezuelae* encodes for 10 putative cyclic di-GMP-metabolizing enzymes but the cellular processes controlled by the cyclic di-nucleotide remained elusive.

**Objectives**

Aiming to gain more knowledge about the regulation of the developmental processes in *S. venezuelae*, we found that elevated levels of c-di-GMP lead to a pronounced delay of the developmental program. On the other hand, depletion of c-di-GMP results in an enhanced sporulation phenotype due to bypassing the aerial mycelium stage.

**Methods**

We applied a global pull-down approach using the c-di-GMP Capture Compound (Caprotec, Berlin) and found a c-di-GMP-responsive regulator that connects c-di-GMP signalling pathways and developmental processes in *S. venezuelae*. Using structural and biochemical analyses we identified a previously unseen oligomeric form of c-di-GMP, which enables the effector protein to dimerize by binding to a heretofore unknown binding motif.

**Conclusions**

Altogether, we demonstrate that the signalling molecule c-di-GMP controls the hypha-to-spore transition in filamentous bacteria.

**FEMS-2876**

## **Bacterial persistence and Toxin - Antitoxins**

### **Magic Spot Controls Bacterial Persister Cell Formation by Activating Toxin - Antitoxins**

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#### **Background**

We showed previously that multidrug tolerance (persistence) of the model organism *Escherichia coli* K-12, depends stochastic induction of Toxin - Antitoxin (TA) activity by (p)ppGpp in a regulatory cascade that included also Lon protease and polyphosphate (Maisonneuve et al., **Cell**, 154, 1140-50, 2013). The TAs involved all encode inhibitors of translation, such as RelE, MazF, HicA etc. Remarkably, all antitoxins of *E. coli* K-12 encoded by type II TA loci are degraded by Lon that, in turn, is activated by polyphosphate. The model explaining stochastic induction of persistence is shown in **Figure 1**. Sophie Helaine and David Holden (Imperial College London, UK) showed that a similar mechanism allows cells of *Salmonella* to survive antibiotic treatment within macrophages (**Science** 343, 204–208, 2014).

#### **Objectives**

Many bacterial pathogens contain multiple type II TA genes and rely on (p)ppGpp to be virulent (**Figure 2**). We will determine the role(s) that (p)ppGpp and TAs play in persistence and virulence of different bacterial pathogens, including, *Mycobacterium tuberculosis*, *Burkholderia cenocepacia* and *Photobacterium luminescence*.

#### **Methods**

In this work, we will employ physiological, genetic, cytological and biochemical methods to reach our aim.

#### **Conclusions**

Preliminary results indicate that indeed (p)ppGpp is required for persistence of bacterial pathogens.

**FEMS-3162**

**Bacterial persistence and Toxin - Antitoxins**

**FIC domains in *Bartonella*: Evolution of diversified host effectors from a widely-spread bacterial toxin/antitoxin system**

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The FIC domain being present in thousands of proteins and found in all domains of life mediates post-translational modification of target proteins, typically by transfer of an AMP moiety from ATP onto a target tyrosyl or threonyl side chain by a process called AMPylation or adenylation. The alpha-proteobacterial genus *Bartonella* comprises facultative intracellular pathogens that utilize a VirB type IV secretion system to translocate a cocktail of bacterial effectors protein into host cells in order to subvert cellular function to the benefit to the bacterial intruder. Diversified copies of the FIC domain are prominently presented in these effector sets, indicating that the various FIC domain variants may mediate diverse effector functions during infection. I will discuss the evolutionary origin of the FIC domain as a bacterial toxin-antitoxin system, its fusion with a type IV secretion signal to constitute an ancestral translocateable effector, as well as the duplication and diversification of the ancestral effector gene in the course of the evolution of the effector cocktails that facilitate host cell infection by bartonellae in diverse mammals.

**FEMS-0655**

**Bacterial persistence and Toxin - Antitoxins**

**Novel regulatory mechanisms in toxin-antitoxin modules**

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Prokaryotic toxin-antitoxin modules are involved in the establishment of persister cells. The latter involves complex regulatory mechanisms that link the regulation of protein activity to regulation of transcription. Recent structural and biochemical data shows a plethora of molecular mechanisms to achieve this goal. In the *phd/doc* module, conditional co-operativity is established via a combination of negative co-operativity through entropic exclusion of and IDP domain combined with a low to high affinity switch for the interaction between toxin and antitoxin. While Doc stabilizes the DNA binding conformation of Phd, Phd likewise inhibits the kinase activity of Doc and simultaneously prevents misfolding of Doc. The same phenomenon of conditional co-operativity is observed for *mazEF* and *ccdAB*, but with a different structural basis, although intrinsic disorder in the antitoxin is a common theme. Other TA modules such as *higBA* are regulated through a seemingly more simple mechanism where the antitoxin acts as the sole repressor, while toxin significantly weakens operator binding. Such a mechanism is observed in several *higBA* modules as well as in *mqsRA* and allows for transcription regulation without the specific need of intrinsic disorder.

**FEMS-2376**

**Bacterial persistence and Toxin - Antitoxins**

**Chaperone-mediated control of toxin-antitoxins**

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Bacterial toxin-antitoxin systems are stress-responsive elements generally composed of a stable toxin that forms an inactive complex with its less stable cognate antitoxin. In response to specific stress conditions the antitoxin is degraded by stress proteases and the free active toxin subsequently targets important cellular processes such as DNA replication or protein synthesis. It is believed that the resulting growth inhibition facilitates adaptation to stress and persistence. *Mycobacterium tuberculosis*, the causing agent of human tuberculosis, encodes 79 putative toxin-antitoxin systems and it has been proposed that persistence induced by active toxins might be relevant for its pathogenesis. The tripartite toxin-antitoxin-chaperone (TAC) system of *M. tuberculosis* is an atypical toxin-antitoxin system strongly induced in persisters and tightly controlled by the molecular chaperone Rv1957, which is related to the canonical SecB chaperone involved in Sec-dependent protein export in Gram negative bacteria. Indeed, in spite of very little sequence similarity, Rv1957 is able to efficiently replace SecB during protein export in *E. coli*, and to specifically control the functional HigB1-HigA1 toxin-antitoxin system of *M. tuberculosis*. How does the mycobacterial SecB-like chaperone respond to stress and control the toxin activation cascade, and to what extent such activation is important for *M. tuberculosis* persistence and virulence are so far unresolved questions.

In this work, we will present recent data concerning the molecular mechanism of TAC complex formation and toxin activation, with emphasis on the role played by the SecB-like chaperone.



**FEMS-1011**

**CRISPR - biological and technological advances**

**Type II CRISPR-Cas9 systems: mechanisms, evolution and applications**

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The RNA-programmable CRISPR-Cas9 system has recently emerged as a transformative technology in biological sciences, allowing rapid and efficient targeted genome editing, chromosomal marking and gene regulation. In this system, the endonuclease Cas9 or catalytically inactive Cas9 variants are programmed with single guide RNAs (sgRNAs) to target site-specifically any DNA sequence of interest given the presence of a short sequence (Protospacer Adjacent Motif, PAM) juxtaposed to the complementary region between the sgRNA and target DNA. The system is efficient, versatile and easily programmable.

Originally, CRISPR-Cas is an RNA-mediated adaptive immune system that protects bacteria and archaea from invading mobile genetic elements (phages, plasmids). Short crRNA (CRISPR RNA) molecules containing unique genome-targeting spacers commonly guide Cas protein(s) to invading cognate nucleic acids to affect their maintenance. CRISPR-Cas has been classified into three main types and further subtypes. CRISPR-Cas9 originates from type II CRISPR-Cas that has evolved unique molecular mechanisms for maturation of crRNAs<sup>a</sup> and targeting of invading DNA<sup>a,b</sup>, which we identified in the human pathogen *Streptococcus pyogenes*. On the basis of the discovery of the DNA targeting mechanism, we proposed that RNA-programmable Cas9 could offer considerable potential for genome editing in cells of the three kingdoms of life for biotechnological, biomedical and gene-therapeutic purposes<sup>b</sup>. As demonstrated by a large number of studies published in the last 18 months, DNA targeting by CRISPR-Cas9 has quickly been adopted by the scientific community to edit and silence genomes in a large variety of cells and organisms including human cells, plants and mice. I will discuss the biological roles of CRISPR-Cas9, the mechanisms involved, the evolution of type II CRISPR-Cas components in bacteria and the applications of CRISPR-Cas9 as a novel genome engineering technology.

**FEMS-3154**

**CRISPR - biological and technological advances**

**Specific nucleic acid targeting by the Type III CRISPR-Cas complexes**

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CRISPR-Cas is a prokaryotic adaptive immune system that provides sequence-specific defense against foreign nucleic acids. Comparative analysis has revealed at least 3 distinct types of CRISPR-Cas systems. During the past few years, much has been learned on the structures and mechanisms of the effector complexes of Type I (Cascade) and Type II (Cas9). Until recently, structural and functional insights on the effector complexes of Type III CRISPR-Cas systems has lagged behind. To address this, the Type III-A (Csm) and III-B (Cmr) complexes of *Thermus thermophilus* have been selected as model systems. Integrated structural analyses (MS, EM) have revealed that the overall architecture of both complexes strongly resembles that of the Type I Cascade complexes. TtCsm is composed of five different protein subunits (Csm1-Csm5), whereas TtCmr has six subunits (Cmr1-Cmr6). Like Cascade, both Type III complexes have an uneven stoichiometry and a single crRNA guide. Interestingly, the TtCsm crRNA content is similar to the Type III-B Cmr complex, indicating that crRNAs are shared among different subtypes. Unexpectedly, both TtCmr and TtCsm cleave complementary target RNAs at multiple sites. Outstanding questions of the *Thermus* Type III systems include (i) a motif for auto-immunity protection (PAM analog), (ii) some target quality control (seed analog), and (iii) last but not least the potential of RNA-guided DNA targeting. The latest insights in the TtCsm and TtCmr mechanisms will be discussed.

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Taylor, D.W., Zhu, Y. et al. (2015) in press

**FEMS-2906**

**CRISPR - biological and technological advances**

**Memory formation in E. coli CRISPR-Cas systems**

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An adaptive immune system in prokaryotes called CRISPR (clustered regularly interspaced short palindromic repeats) uses small guide RNAs to neutralize invading viruses and plasmids. In *Escherichia coli*, immunity depends on a ribonucleoprotein complex called Cascade. This protein complex recognizes double-stranded DNA from invaders by forming basepairs between the guide RNA and invader DNA. The structure of Cascade displays an unusual seahorse-shape that binds target DNA in an unique underwound ribbon like duplex. This recruits the effector nuclease Cas3 which progressively degrades the target DNA in an ATP-dependent manner, neutralizing the virus infection. Apart from giving insight into the crystal structure and function of Cascade, I will also show how viruses are able to escape immunity by point mutagenesis, and unveil how hosts rapidly adapt their immune specificity making use of prior memories to effectively counter escape mutants.

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Brouns SJJ, Jore MM, Lundgren M, Westra ER, Slikhuis RJ, Snijders APL, Dickman MJ, Makarova KS, Koonin EV, Van der Oost J. Small CRISPR RNAs guide antiviral defense in prokaryotes. **Science**. 2008 Aug 15;321(5891):960-4.

**FEMS-1197**

**CRISPR - biological and technological advances**

**Studying and fighting bacteria with the help of CRISPR**

## **FEMS-0950**

### **Establishment of the fungal mycelium**

#### **Heterogeneity of the fungal mycelium**

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Fungi form colonies that consist of a network of hyphae. Macro-colonies with a diameter > 1 cm are formed on solid media, while micro-colonies with a diameter in the mm-scale are formed in liquid shaken cultures such as bioreactors. Research in our lab has revealed that micro- and macro-colonies are not a collection of hyphae with a synchronized activity. Fungal micro-colonies within a liquid culture are heterogeneous in gene expression and size. Heterogeneity in RNA composition can even be found between and within zones of macro- and micro-colonies. These findings imply that gene regulatory networks will not be correctly predicted by using whole cultures and colonies. This will be illustrated by regulatory networks of amylolytic genes and genes involved in asexual development. The finding of heterogeneity came to a surprise considering the fact that hyphae of higher fungi are compartmentalized with septa that contain large pores. These pores enable cytoplasmic streaming. It has now become clear that cytoplasmic streaming in aspergilli is reduced by dynamic plugging of septa. Modelling has shown that even open septa are a barrier for cytoplasmic mixing.

**FEMS-2786**

**Establishment of the fungal mycelium**

**The microtubule cytoskeleton in *Aspergillus nidulans***

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Polar growth in fungi requires intact microtubule and actin cytoskeletons. Both cytoskeletons consist of filamentous structures composed of alpha/beta tubulin dimers and actin monomers, respectively. The formation of both cytoskeletons is interdependent and relies on a class of so called cell-end marker proteins. The organization of the two cytoskeletons guarantees the directed travel of secretory vesicles. We found in *Aspergillus nidulans* that hyphal extension occurs in pulses at small areas at the hyphal tip rather than uniformly. Sites of secretion are only maintained for a short period before new sites are established.

*A. nidulans* harbors two genes encoding alpha tubulin and two genes encoding beta-tubulin. However, 2-D protein analyses revealed early on that tubulin can be post-translationally modified. Such modifications are a common phenomenon in eukaryotes, but their role is largely unknown. One modification is the C-terminal removal of tyrosin from alpha tubulin. The missing tyrosin can be re-added to alpha/beta tubulin dimers. Hence there is a cycle of detyrosination and tyrosination which determines the amount of modified alpha tubulin. Such modifications have not yet been described in fungi. We found that the kinesin 3 motor UncA binds preferentially to a subpopulation of microtubules in *A. nidulans* and also in *N. crassa*. We have evidence that the subpopulation consists of detyrosinated alpha tubulin and that the tail of UncA is required for the specificity. However, recent results suggest that the UncA tail harbours a microtubule-binding site and that the motor can interact with the motor domain with one microtubule and with the tail with a adjacent one. Thus UncA could be involved in bundling of microtubules.

**FEMS-2352**

**Establishment of the fungal mycelium**

**Signaling cascades associated with fungal cell-cell communication and trophic growth**

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Intercellular communication is critical for the survival of unicellular microbes as well as for the development and function of multicellular tissues in higher eukaryotes. Cell-to-cell signaling is also required to develop the interconnected mycelial network characteristic of filamentous fungi and is a prerequisite for symbiotic and pathogenic host colonization achieved by molds. Somatic cell-cell communication and subsequent cell fusion is governed by the MAK-2 mitogen activated protein kinase (MAPK) cascade in the filamentous ascomycete model *Neurospora crassa*, yet the composition and mode of regulation of the MAK-2 pathway are currently unclear. In order to identify additional components involved in MAK-2 signaling we performed affinity purification experiments coupled to mass spectrometry with strains expressing functional GFP-fusion proteins of the MAPK cascade. This approach identified STE-50 as regulatory subunit of the yeast Ste11 homolog NRC-1 and HAM-5 as cell-communication-specific scaffold protein of the MAPK cascade. Moreover, we defined a network of proteins consisting of two Ste20-related kinases, the small GTPase RAS-2 and the adenylate cyclase capping protein CAP-1 that function upstream of the MAK-2 pathway and whose signals converge on the NRC-1/STE-50 MAP3K complex and the HAM-5 scaffold. Finally, our data imply the COT-1 NDR kinase network as effector module of the MAK-2 pathway to control the chemotrophic behavior of the two communicating cell tips. Taken together, these data have implications for our mechanistic understanding of MAPK pathways and their regulation during intercellular communication in eukaryotic microbes.

**FEMS-1744**

**Establishment of the fungal mycelium**

**Insights into the early regulation of conidiospore development in *Aspergillus nidulans***

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Fungal hyphae are capable of undergoing different developmental programmes, thus enabling the mycelium to perform diverse biological functions simultaneously.

*Aspergillus nidulans* mycelia expand by means of vegetative hyphae at the colony periphery. At sub-peripheral regions, hyphae give rise to conidiophores bearing aerial mitospores (conidiospores) suitable for dispersal. At distal regions, hyphae support cleistothecium formation and eventually meiospores (ascospores), with long-term survival capabilities. The relative proportions of these cell-types can be further modulated to suit environmental conditions.

Three decades of molecular genetic research have unveiled the most important factors participating in the regulation of conidiophore development. Those already expressed in vegetative cells prior to the onset of development have been shown to participate in cell fate determination. Moreover, their characterisation has revealed that protein-protein interactions between these factors and their counterparts for cleistothecium formation, into protein complexes, are at the core of cell fate determination. Indeed, the alternative patterns of complex formation may well determine the alternative patterns of development.

In this presentation, we review the interaction patterns described for the velvet family of proteins VeA, VelB, VosA and VelC, the red light photosensor FphA, and the blue light photosensors LreA and LreB. We shall also incorporate interactions involving FluG and FlbA-E, developmental regulators specifically associated with conidiophore formation. An updated model of interactions leading to the determination of alternative developmental programmes will be presented.



## **FEMS-2970**

### **Thermodynamics in ecology and systems biology**

#### **231 years after Lavoisier, in times of systems ecology – what tells us biological calorimetry?**

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Heat is an unavoidable side-product of all life processes. Already in 1784 reported the father of quantitative chemistry – Antoine Laurent de Lavoisier – on the first measurement of the metabolic heat of a guinea pig and made first attempts to offer an interpretation. The main advantages of heat as an indicator for life processes are the reflection of any metabolic changes in real time and the tight correlation to metabolic fluxes and thermodynamic driving forces. Nowadays, even commercially available calorimetric instruments achieve detection limits low enough to measure the heat of 100 000 aerobically growing bacteria or of 100 myocardial cells. Heat can be monitored in reaction vessels ranging from a few nanoliters up to many cubic meters. In contrast to many traditional and emerging microbiological methods, heat flux measurements do not need labeling agents and reactants and also works with optically opaque solutions. It is further possible to assemble the thermal transducer in a protected way that reduces aging and thereby signal drifts.

All of these advantages make calorimetry an interesting tool for many applications in medicine and biotechnology. Unfortunately, metabolic heat signals are currently mainly used to monitor and control biological processes and their full information potential remains unexploited. However, new omics technologies offer a flood of information about complex biological and ecological systems. These detail information open the chance to be combined with calorimetric measurements and thermokinetic modelling to provide a holistic picture of the complex system under consideration. Various methods for quantitative data interpretations and their dependency on the applied calorimetric device and available additional biometric data will be presented and discussed. Also potential misinterpretations are considered.

## **FEMS-0603**

### **Thermodynamics in ecology and systems biology**

#### **A novel biochemical trick of anaerobic microorganisms to deal with complicated thermodynamic situations**

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Flavin-based electron-bifurcation is a recently discovered mechanism of coupling endergonic to exergonic redox reactions in the cytoplasm of anaerobic bacteria and archaea (1-7). The seven electron-bifurcating complexes characterized to date have in common that they catalyze the reduction of ferredoxin and of a second electron acceptor with NADH, NADPH, dihydrogen or formate as electron donors, the latter having a redox potential between that of ferredoxin and of the second electron acceptor. Via this mechanistic "trick" low potential electrons are generated that are used for energy conservation and biosyntheses in strict anaerobe such as Clostridia, acetogenic bacteria, methanogenic archaea and sulfate reducing bacteria.

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## FEMS-2308

### Thermodynamics in ecology and systems biology

#### **A thermodynamic theory of microbial growth and its perspectives for a better understanding of community dynamics**

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The study of microbes, the simplest form of life, constitutes a fertile thinking ground for a deeper interlinking between physical and biological concepts. In 1922, Alfred Lotka suggested that the similarity between individuals was an invitation to imagine a "statistical mechanics of living beings"<sup>1</sup>. As an extension of these ideas, we proposed a thermodynamic theory of microbial growth by showing how systems constituted by microbes in contact with molecules could be likened to ensembles described by the laws of statistical physics. Based on thermodynamic balances established by different authors<sup>2</sup>, we defined the "activation energy" of a microbe and thus the probability for an elementary division act to be triggered. A growth equation could be proposed<sup>3</sup>, which links a flux (the growth of microbes) to a force (the free energy density in the environment). The equation allows adequate modeling of experimental growth data. More importantly, it also allows making new predictions in relation to the microbial isotopic fractionation phenomenon which can be viewed as a kinetic consequence of the differences in energy contents of isotopic isomers used for growth. We showed how recent experimental data actually support these original predictions. In the future, we plan to use our thermodynamic growth equation for modelling microbial community dynamics and studying the convergence of microbial ecosystems. Indeed our law offers a mathematical framework that may help understand the relevance of thermodynamic ecological goal functions such as dissipated power, exergy or entropy production.

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**FEMS-2806**

**Computational modelling**

**Mechanistic basis of equal plasmid spacing by the parABC system**

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<sup>1</sup>*Computational and Systems Biology, John Innes Centre, Norwich, United Kingdom*

Low copy number plasmids in bacteria require segregation for stable inheritance through cell division. This is often achieved by a *parABC* locus, comprising an ATPase ParA, DNA-binding protein ParB and a *parC* region, encoding ParB-binding sites. These minimal components space plasmids equally over the nucleoid, yet the underlying mechanism is not understood. Here we investigate a model where ParA-ATP can dynamically associate to the nucleoid and is hydrolyzed by plasmid-associated ParB, thereby creating nucleoid-bound, self-organizing ParA concentration gradients. We show mathematically that differences between competing ParA concentrations on either side of a plasmid can specify regular plasmid positioning. Such positioning can be achieved regardless of the exact mechanism of plasmid movement, including plasmid diffusion with ParA-mediated immobilization or directed plasmid motion induced by ParB/*parC*-stimulated ParA structure disassembly. However, we find experimentally that *parABC* from *Escherichia coli* plasmid pB171 increases plasmid mobility, inconsistent with diffusion/immobilization. Instead our observations favor directed plasmid motion. Our model predicts less oscillatory ParA dynamics than previously believed, a prediction we verify experimentally. We also show that ParA localization and plasmid positioning depend on the underlying nucleoid morphology, indicating that the chromosomal architecture constrains ParA structure formation. Our directed motion model unifies previously contradictory models for plasmid segregation and provides a robust mechanistic basis for self-organized plasmid spacing that may be widely applicable.

Ietswaart R, Szardenings F, Gerdes K, Howard M (2014) Competing ParA Structures Space Bacterial Plasmids Equally over the Nucleoid. PLoS Comput Biol 10(12): e1004009. doi:10.1371/journal.pcbi.1004009

**FEMS-2841**

**Computational modelling**

**The role of gene expression noise in the evolution of gene regulation**

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Although it is often tacitly assumed that gene regulatory interactions are finely tuned, it is unclear how accurate gene regulation could evolve de novo from a state without regulation. Moreover, gene expression noise would seem to impede the evolution of accurate gene regulation, and the fact that expression noise varies greatly across genes suggests natural selection has affected noise levels. To infer how selection has tuned noise levels of *E. coli* promoters, we designed a novel synthetic biology approach in which we evolved a large library of synthetic promoters de novo. We show that, surprisingly, promoters exhibit low noise by default, and rather than having acted to lower noise levels, selection must have acted to raise noise levels of a substantial fraction of *E. coli* promoters. In addition, promoters with elevated noise levels are precisely those that are highly transcriptionally regulated.

To explain the general association between expression noise and gene regulation, it has been argued that elevated expression noise may either be directly selected as a 'bet hedging strategy', or that it may be a detrimental but unavoidable side-effect of regulation. We here present a general theory for the interplay of expression noise and gene regulation which shows that this is a false dichotomy: expression noise can be both a necessary consequence of regulation and a beneficial bet hedging strategy at the same time. Moreover, this theory elucidates how regulation can evolve incrementally starting from a state without regulation. Noise propagation from regulators to targets acts as a rudimentary form of regulation, and noisy expression is not a nuisance but rather a stepping stone toward the emergence of accurate gene regulation.

**FEMS-2844**

**Computational modelling**

**Stochasticity and homeostasis in cellular growth**

S. Tans<sup>1</sup>

<sup>1</sup>*Systems Biophysics, AMOLF institute, Amsterdam, Netherlands*

Elucidating the role of molecular stochasticity in cellular growth is important to understanding phenotypic heterogeneity and the stability of cellular proliferation. We used time-lapse microscopy to measure fluctuations in the instantaneous growth rate of single cells of *Escherichia coli* and in the expression of metabolic enzymes. We show that expression fluctuations of catabolically active enzymes can propagate and cause growth fluctuations. Conversely, growth fluctuations propagate back to perturb expression. Homeostasis is promoted by a noise-cancelling mechanism that exploits fluctuations in the dilution of proteins by cell-volume expansion. Thus, molecular noise is propagated not only by regulatory proteins but also by metabolic reactions. The results suggest that cellular metabolism is inherently stochastic, and a generic source of phenotypic heterogeneity.

**FEMS-3158**

**Computational modelling**

**How bacteria can maximise their growth rate under stress conditions**

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Large population-sizes and the exponential increase in the abundance of fit genotypes makes growth-rate selection a powerful force that shapes bacterial survival strategies. Bacteria have to therefore carefully balance growth and stress tasks to prevent being outcompeted during nutrient-excess and stress conditions. In this talk, I will discuss several principles of bacterial-fitness optimisation, starting from basic relations of biochemistry, steady-state metabolism and growth-rate maximisation. The resulting theory explains how bacteria should balance growth and stress tasks to optimise fitness. Using this theory, several experimental findings can be explained. Those findings include how *E. coli* regulates its ribosome synthesis via ppGpp, the fitness costs of protein overexpression and the shape of fitness landscapes.

**FEMS-0703**

**White biotechnology**

**Bio-synthetic routes based on new enzymatic reactions**

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Traditionally, new enzyme activities have been discovered by screening microorganisms and mutant libraries. In recent years, these strategies were successfully expanded by a mechanism-based approach. Hereby, the catalytic machinery of known enzymes is harnessed to perform not only the same type of chemistry with different substrates but also to catalyze completely new non-physiological reactions. To identify initial activity and to address the catalytic flexibility, small and functionally rich enzyme libraries have been used. This presentation will highlight hidden as well as uninvestigated enzymatic activities and will illustrate strategies to unmask them. For example, we have recently harnessed the unique protonation machinery of squalene hopene cyclases to generate enzymes for non-natural chiral Brønsted acid catalysis.

The identification of novel enzymatic transformations enables complete new biosynthetic routes to interesting molecules. Examples like menthol, sebacic acid or heterocyclic compounds will be given which also explains how these new reactions can be applied in a retrosynthetic approach.



**FEMS-1219**

**White biotechnology**

**Microbial biosynthesis and separation of alkanes and alcohols**

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**Microbial biosynthesis and separation of alkanes and alcohols**

Economically sustainable conversion of solar energy and CO<sub>2</sub> into low value chemical energy (e.g. fuel) using biotechnology is a challenge. Several benefits can be imagined if the chemical product can be immediately separated from the (1) organisms that synthesized the product and (2) biotechnological process. These include enhanced product tolerance, reduced feedback inhibition, and enhanced pathway thermodynamics. In addition, by continuously removing the product, a continuous biotechnological process is possible. This allows the biological organism to act as a catalyst rather than as a substrate for further transformation. Several strategies for immediate product-process separation are possible: (a) a gaseous product spontaneously leaves the liquid phase, (b) excretion and engineered separation of a water-soluble liquid, (c) excretion and spontaneous separation of a hydrophobic liquid. In all above cases, it is necessary for the product to transfer through the cell envelope of the biocatalyst. Towards the objective of producing a useful fuel that can be easily separated, we have constructed several synthetic pathways for alkane and alcohol biosynthesis. The challenges and insight gained from this metabolic pathway and transporter engineering work will be summarised.

**FEMS-2880**

**Antibiotic resistance: human and veterinary medicine**

**Parallel but distinct evolution of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* toward pan-drug resistance**

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*Pseudomonas aeruginosa* and *Acinetobacter baumannii* have emerged as major nosocomial pathogens in the second half of the 20<sup>th</sup> century, as unexpected consequence of medical progress. These strictly aerobic bacilli share a high metabolic versatility and the ability to thrive in hostile environments. In addition to producing a complex array of virulence factors enabling them to infect fragile patients, both pathogens show a remarkable capacity to become resistant to all the antibiotics available. However, despite these apparent similarities, *P. aeruginosa* and *A. baumannii* differ in the mechanisms leading to such a high degree of resistance. Total recalcitrance of *P. aeruginosa* to  $\beta$ -lactams, the major class of antibiotics used in therapeutics, mostly relies on mutation-driven mechanisms (overproduction of intrinsic  $\beta$ -lactamase AmpC, porin OprD deficiency, activation of efflux systems) operating synergistically with a variety of extended-spectrum  $\beta$ -lactamases collected from environmental reservoirs via horizontal gene transfers. The parallel evolution of *A. baumannii* to pan- $\beta$ -lactam resistance involves IS-dependent up-regulation of natural  $\beta$ -lactamase ADC combined with conjugational acquisition of a limited number of OXA-type  $\beta$ -lactamases from closely-related species. Accumulating evidence suggests that the two microorganisms exploit distinct pools of resistance genes (resistomes) to reinforce their own resistance mechanisms, probably as a result of distinct ecological niches.

**FEMS-2835**

**Antibiotic resistance: human and veterinary medicine**

**Antimicrobial resistance: a touchy interface between human and veterinary medicine**

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<sup>3</sup>*Enteric Bacteriology and Foodborne Zoonoses 3Scholarship of János Bolyai of H.A.  
S.,*

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Academy of Sciences (H.A.S), Budapest, Hungary*

The increasing challenge due to antimicrobial resistance of pathogenic bacteria in human-, and veterinary medicine prompted the general fear that several classes of antimicrobials are becoming more or less useless due to their prophylactic and/or therapeutic use for animals. In light of new knowledge on genomic flexibility and on clonal spreading of pathogens and/or commensal bacteria we aim to re-visit potential links of antimicrobial resistance between animals and man.

Data about possible ways of evolution of resistances against the most critical antimicrobials (third generation cephalosporins, fluoroquinolones and aminoglycosides) show that foodborne enteric pathogens like *Salmonella* and *Campylobacter* seem to be trafficking frequently from animals to man, with the occasional opportunity to transfer critical resistance determinants. In contrast to enteric pathogens, the dominant clones of non-enteritis causing antibiotic resistant bacteria - methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus faecium*, multiresistant *Pseudomonas aeruginosa* - usually differ substantially in the human healthcare setting from those occurring in animals.

Adaption of distinct clones of bacteria to various species of animals or to humans may have contributed to the observed clonal diversity. The use of fluoroquinolone type antibiotics could also emerge as an enhancer of major international clones of MRSA and of some additional multiresistant pathogens.

Thus, evolution and spread of resistant enteric isolates is frequently driven by the development of some major clones originating and spreading from/within human clinical settings, while the resistant clones of animal/food origin seem to be more diverse with a partial human overlap. Therefore, the differences in spreading antimicrobial resistance within and between differing epidemiological compartments of animals and humans should be considered more analytically.

**FEMS-1735**

**Antibiotic resistance: human and veterinary medicine**

**A NOVEL D-ALANINE-D-ALANINE LIGASE GENE FROM HUMAN ORAL METAGENOMIC DNA CONFERS HIGH-LEVEL RESISTANCE TO D-CYCLOSERINE**

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**Background**

The human oral cavity is a reservoir for antibiotic resistance genes. While investigating integrons (a natural gene capture and expression system) and their associated gene cassettes from the human oral cavity, we identified two variants of D-alanine-D-alanine ligase (*ddl*) predicted to encode novel variants of the protein. The Ddl enzymes catalyse the synthesis of D-Ala-D-Ala dipeptide during the early stage of peptidoglycan biosynthesis. D-Cycloserine (DCS) is a Ddl inhibitor and is used as a second-line antibiotic to treat multidrug-resistant and extensively drug-resistant *Mycobacterium tuberculosis*. Overexpression of *ddl* genes from either *Mycobacterium smegmatis* or *M. tuberculosis* has been shown to confer resistance to DCS in *M. smegmatis*.

**Objectives**

The aim of this study is to test the hypothesis that *ddl*, identified on integron gene cassettes from a human oral metagenome may confer resistance to DCS.

**Methods**

Minimum Inhibitory Concentration (MIC) of DCS in *Escherichia coli* and *Bacillus subtilis* overexpressing the *ddl* variants was determined by agar dilution method according to the guidelines of Clinical and Laboratory Standards Institute (CLSI).

**Conclusions**

We observed that one variant of *ddl* confers resistance to DCS in *E. coli* and *B. subtilis* with an MIC of 128 µg/ml and 256 µg/ml, respectively. The other variant confers resistance with an MIC of 64 µg/ml and 256 µg/ml, respectively.

These results represent the first time that *ddl* has been found on an integron cassette suggesting that it is important in host adaptation to stress (e.g. cell wall targeting antibiotics). The mechanism by which Ddl confers DCS resistance is currently being characterised.



**FEMS-2646**

**Antibiotic resistance: human and veterinary medicine**

## **GUT RESISTOME DEVELOPMENT IN NEONATES MONITORED IN A COHORT STUDY**

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E. Hamelmann<sup>2</sup>, S. Lau<sup>2</sup>, K. Gerhold<sup>2</sup>, P.F.G. Wolfs<sup>1</sup>

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<sup>2</sup>University Children's Hospital St Josef, Ruhr-Universität Bochum, Bochum, Germany

### **Background**

The antibiotic resistance of pathogenic bacteria has been studied extensively. However, recently the collection of antibiotic resistance genes (ARGs) in the gut microbiota (resistome) has been acknowledged as an important reservoir of ARGs. The establishment of the human gut resistome is ideally studied in the pediatric microbiota.

### **Objectives**

Our objective was to investigate the presence of various ARGs in the microbiota of newborns in the weeks following birth.

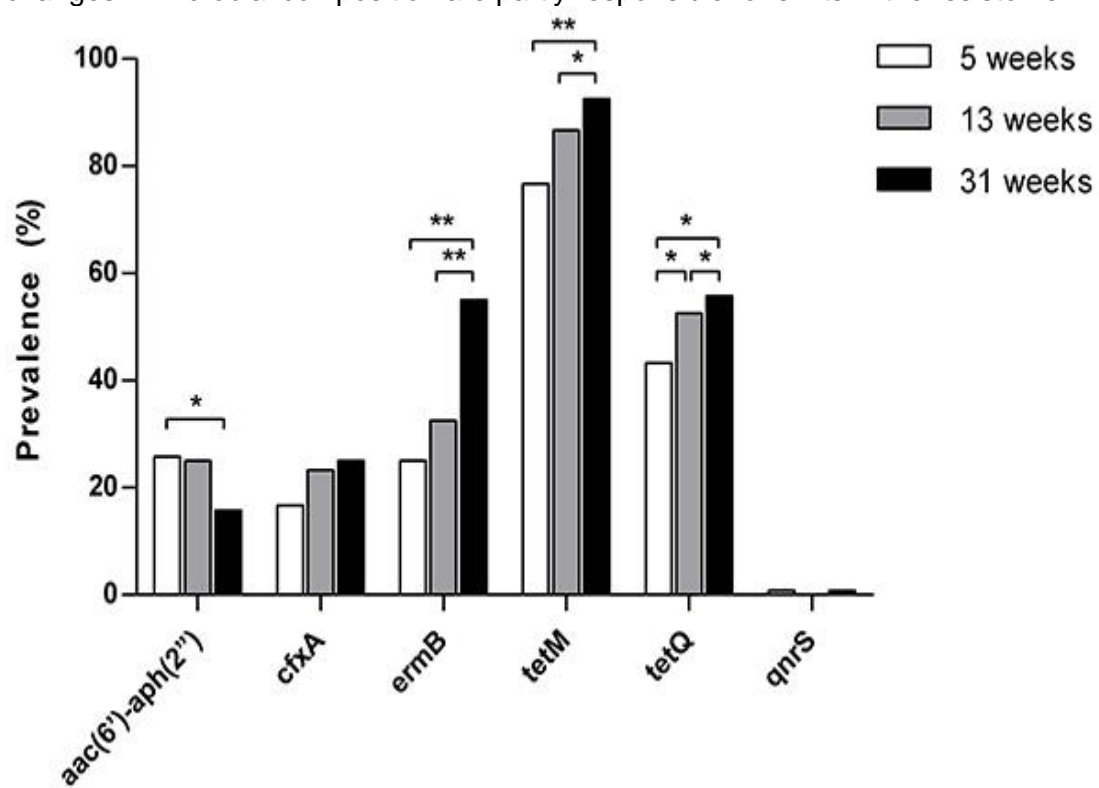
### **Methods**

Within a prospective cohort among neonates born between 2000-2007, fecal samples collected at the ages of 5, 13 and 31 weeks postpartum were analyzed from a random selection of 120 infants. Inclusion criteria included healthy newborns at term, birth weight of  $\geq 2500$ g, and informed consent. Fecal samples were subjected to targeted metagenomics by qPCR for the detection of ARGs *cfxA*, *tetM*, *tetQ*, *aac-aph*, *ermB* and *qnrS*. The microbial composition in all samples was determined in a previous study. Prevalence was compared by using a McNemar's test for paired samples. Risk factors were analyzed by using logistic regression.

### **Conclusions**

Results show that acquisition of ARGs in the gut microbiota occurs already within the first weeks after birth and that the prevalence of these genes in the microbiota of newborns is highly variable over the course of a few weeks (Figure). Mode of delivery as well as duration of breastfeeding brought about significant changes in the prevalence of ARG. Relating ARG prevalence to microbiome analysis indicated that

changes in microbial composition are partly responsible for shifts in the resistome.



**FEMS-0564**

**Antibiotic resistance: human and veterinary medicine**

**MOLECULAR CHARACTERISTICS OF EXTENDED-SPECTRUM  
CEPHALOSPORIN-RESISTANT ENTEROBACTERIACEAE FROM HUMANS IN  
THE COMMUNITY DIFFER FROM THOSE IN BROILERS**

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**Background**

ESBL/AmpC-producing Enterobacteriaceae have frequently been reported in broilers and therefore they have been considered as a reservoir for ESBL/AmpC-encoding resistance genes with the potential to transmit to humans.

**Objectives**

The objective was to investigate the molecular characteristics of extended-spectrum cephalosporin (ESC)-resistant Enterobacteriaceae collected during a cross-sectional study examining the prevalence of faecal carriage of extended-spectrum  $\beta$ -lactamase (ESBL)-producing Enterobacteriaceae in a population sample of humans living in areas with high or low broiler density.

**Methods**

ESC-resistant Enterobacteriaceae were identified by combination disc-diffusion test. The presence and composition of ESBL/AmpC/carbapenemase encoding genes were assessed by PCR and sequencing. For *Escherichia coli*, phylogenetic groups and MLST were determined.

**Conclusions**

175 ESC-resistant Enterobacteriaceae were cultured from 165 of 1,033 sampled persons, yielding a prevalence of 15.9 %. No carbapenemase genes were identified. Most isolates were *Escherichia coli* followed by *Citrobacter freundii* and *Enterobacter cloacae*. The predominant ESBL genes were *bla*<sub>CTX-M-1</sub>, *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-14</sub>, but other allelic variants were also identified as well *bla*<sub>SHV-12</sub> and *bla*<sub>TEM-52</sub>. The most common AmpC genes were *bla*<sub>CMY-2</sub> and *bla*<sub>CMY-48-like</sub>. A large variety of *E. coli* genotypes was found, ST131 and ST10 being most common. ESBL/AmpC genes in Enterobacteriaceae obtained from the community resembled those found in isolates from patients in Dutch hospitals, indicating that healthy humans act as a reservoir for transmission of these determinants to vulnerable people. In contrast, the molecular characteristics of *E. coli* isolates differed from those of broilers suggesting that



broilers are not an important source of ESBL/AmpC genes for humans living in the community.

**FEMS-1715**

**Antibiotic resistance: human and veterinary medicine**

**TRACKING IS26 MEDIATED IN VIVO GENE-SHUFFLING EVENTS ORIGINATING FROM A COMPLEX MULTI-DRUG RESISTANCE LOCUS**

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**Background**

The insertion element IS26 is known to play a crucial role in the rapid evolution of complex drug resistance loci (CRL) of Gram-negative pathogens by shuffling and/or disseminating clustered drug resistance genes. Until recently, the unique molecular mechanisms involved in the mobilization of DNA fragments flanked by IS26 elements were unknown. A model, describing the generation of diverse types of IS26-derived mobile units has now been established using simple artificially constructed resistance units to support the proposed molecular mechanisms. To the best of knowledge, the impact of IS26-mediated gene reshuffling events on naturally occurring CRLs containing multiple copies of IS26 has never been demonstrated.

**Objectives**

To characterize IS26-mediated laterally mobile resistance subunits that have the potential to originate from a specific complex resistance locus.

**Methods**

Combinations of Illumina and Sanger DNA sequencing, gene cloning and qPCR analytical techniques were used.

**Conclusions**

We identified a 27kb resistance locus (containing seven copies of IS26 and genes conferring resistance to five different classes of clinically relevant antibiotics) from the genome sequence of an *Escherichia coli* isolate belonging to sequence type 405, a major emerging uropathogenic *E. coli* clone implicated in the global dissemination of the *bla*<sub>CTX-M15</sub> gene. We have irrefutably tracked formation of five, of 16 possible, laterally mobile units encoding resistance to different combinations of clinically important antibiotics from the 27kb CRL. Our assays on the CRL documents, for the first time, IS26-mediated horizontal gene mobilisation events *in-vivo* and experimentally confirm the imperative role played by IS26 in rapid evolution of CRL.



**FEMS-0595**

**Microbial transformations of metals and metalloids**

**Ecology and mechanisms of microbial Fe(II) oxidation**

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The two most important redox states of iron in the environment are Fe(II) [ferrous iron] and Fe(III) [ferric iron]. Dissolved Fe(II) and relatively soluble Fe(II) minerals are abundant in many environments including pH-neutral soils, sediments and iron seeps. Oxidation of dissolved and solid-phase ferrous iron [Fe(II)] at neutral pH can be catalyzed by microaerophilic, nitrate-reducing and even phototrophic microorganisms and leads to the precipitation of poorly soluble Fe(III) minerals. These minerals influence the environmental fate of many nutrients and contaminants and the precipitation of Fe(III) minerals by these three microbial processes has been shown to occur in many modern environments and has been suggested to have been present already on early Earth.

This presentation will summarize the current knowledge and show results regarding mechanisms, physiology, and ecology of microbial Fe(II) oxidation. Special focus will be on microaerophilic Fe(II)-oxidizing bacteria that thrive in gradients of ferrous iron and oxygen and on phototrophic and nitrate-reducing Fe(II)-oxidizing bacteria in freshwater and marine sediments. I will present data on the isolation and quantification of microaerophilic, nitrate-reducing and phototrophic Fe(II)-oxidizers from different habitats as well as results regarding the quantification of their activities under different environmental conditions.

**FEMS-2448**

## **Microbial transformations of metals and metalloids**

### **Microbial oxidation of metal sulfur minerals**

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### **MICROBIAL OXIDATION OF METAL SULFUR MINERALS**

Microorganisms can significantly impact oxidation rate of sulfur minerals such as pyrite, sphalerite, galena and arsenopyrite and thus regulate sulfur and metals cycles in various environments. A wide range of acidophilic bacteria and archaea are capable of catalyzing sulfur oxidation near the Earth's surface resulting in production of highly acidic, sulfate-rich solutions known as acid drainage which represents extreme geochemical conditions. Recent molecular ecology studies revealed that extreme geochemical conditions such as acid drainage sites that were previously thought lack of life due to limited nutrients and extreme conditions have diverse microbial populations. These DNA based findings open new directions for research dealing with sulfur oxidation by emphasizing interactions between microbe and sulfur minerals. .

Elucidating the role of microorganism on dissolution of metal sulfide minerals is the key to understand environmental consequences of metal sulfur oxidation and developing of proper remediation techniques. Dissolution and oxidation of metal sulfur minerals usually generates different sulfur species with a wide range of valence states from sulfide (2-) to sulfate (+6). One way to understand metal sulfur oxidation pathway is to use O and S isotopes of reactants and products that are first witness of dissolution reactions. Biotic and abiotic metal sulfide oxidation experiments (pyrite, sphalerite, galena, arsenopyrite and S<sup>0</sup>) was carried out with different *Acidophilic spp.* species under various environmental conditions. Modern analysis techniques in addition to  $\delta^{18}\text{O}$  and  $\delta^{34}\text{S}$  measurements of sulfur species were used to reveal oxidation mechanism (biotic vs. abiotic) of each sulfur mineral. Results will be discussed in detail to step towards understanding of microbial role on the dissolution kinetics of sulfur minerals.

**Key Words:** Enzyme, metal sulfur, isotope, DNA, dissolution

## **FEMS-2048**

### **Microbial transformations of metals and metalloids**

#### **BIOLEACHING OF STRATEGIC ELEMENTS FROM PRIMARY AND SECONDARY RESOURCES**

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#### **Background**

The prices of some economically strategic elements, e. g. indium, germanium, rhenium, vanadium, and antimony, due to their use for high-tech products have increased to an extent which raises the question, whether these elements can be obtained from complex ores or secondary resources also in Germany.

#### **Objectives**

The aim of the studies was to identify bacterial cultures and conditions under which metals mentioned above by microbial activity can be brought into solution. Indium was to be obtained from sphalerite (ZnS) of the ore mountain region around Freiberg, the other strategic metals from "Theisen sludge" which resulted from fine-grained flue dust of former copper smelters and which had been deposited in the Mansfeld area, Germany.

#### **Methods**

For indium from sphalerite molecular analyses of the microbial community of acidic waters in the mine "Reiche Zeche" were performed to identify suitable leaching communities. To obtain other elements from "Theisen sludge" appropriate strains were enriched. All leaching experiments were initially performed in shake flasks in several media by pure or mixed cultures, and compared to abiotic controls. Then bioreactor studies were performed. Redox potential, pH, and metal concentrations were determined.

#### **Conclusions**

Between 80 and 100% of the zinc were leached from sphalerite in 9K and DSM 882 medium by mixed cultures from various sources including "Reiche Zeche" as well as by *Acidithiobacillus ferrooxidans* DSM14882<sup>T</sup>. Due to a co-precipitation of indium with jarosite, an increase in the solids loading resulted in a lower increase of indium as compared to zinc in solution. The other elements mentioned could successfully be leached from "Theisen sludge".

**FEMS-2769**

**Microbial transformations of metals and metalloids**

**BOIFILM CONTRIBUTIONS TO MARINE CORROSION OF 70/30 CUNI ALLOYS**

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**Background**

In marine environments, the activity of microorganisms which form biofilms on metallic materials such as Cu, Fe, Al, and their alloys can result in severe deterioration of these materials, a phenomenon known as microbiologically-influenced corrosion (MIC). It is accepted that biofilms comprising diverse microbial populations are more aggressive towards metals than monoculture biofilms. It has also been proposed that corrosion rates observed in the presence of microbial consortia are related to cooperative metabolic activities of biofilm population, as well as the chemical properties of the colonised surface. As a result of their corrosion resistance, thermal conductivity and reported anti-fouling properties, CuNi alloys find many applications in marine environments and are used extensively in heat-exchangers, pipelines and cladding materials. The stability of these alloys in chloride-containing media has been attributed to the presence of a duplex oxide layer on the alloy surface. However, it has been demonstrated the protective oxide structure on CuNi alloys does not prevent, and merely delay the onset of macrofouling and that these alloys are susceptible to MIC.

**Objectives**

Advanced electron microscopy, surface science and molecular ecology techniques, such as pyrosequencing have been used to characterise the enrichments and resulting biofilms.

**Methods**

Continuous flowing bioreactors operating at 10 C and 24 C have been employed to expose CuNi coupons with different surface pre-treatments to aerobic and anoxic microbial enrichments obtained from marine biofilm samples recovered from corroding and non-corroding systems.

**Conclusions**

The study revealed that there was a correlation between the microbial community structure and the extent of localised corrosion attack.

## FEMS-1701

### Microbial transformations of metals and metalloids

#### CAN WE EXPLOIT BACTERIA AND FUNGI TO PRESERVE ARCHAEOLOGICAL IRON OBJECTS?

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<sup>3</sup>*Haute Ecole Arc Conservation-Restauration, Neuchâtel, Switzerland*

#### Background

Without any conservation-restauration intervention, archaeological iron objects are affected by **dramatic corrosion** that leads to an **irreversible deterioration**. The main issue with this metallic substrate is the chlorine content within the corrosion layers that, reacting with H<sub>2</sub>O and O<sub>2</sub>, causes flacks, cracks and loss of shape of the objects after excavation. Conservation-restauration methods nowadays available are expensive, time consuming and often employ toxic substances.

#### Objectives

Therefore, the MAIA (Microbe for Archaeological Iron Artworks) project aims to develop alternative **conservation-restauration methods for iron artefacts that will be based on the use of microorganisms**.

#### Methods

For this purpose, two metabolic activities of specific microorganisms were studied. First we tested **iron reduction/accumulation by anaerobic bacteria and fungi**, leading to the formation of stable molecules of low molar volume; and second, the capacity of **translocating and/or accumulating chlorine** by fungi.

In order to identify some microorganisms with the desired iron metabolism, two anaerobic bacterial strains of *Desulfitobacterium hafniensis* (LBE and TCE1) and several fungal strains were studied. Different iron sources were tested either as soluble (iron citrate) or solid phases (powdered iron compounds from real objects). Spectrophotometric analyses were carried out to ascertain iron reduction and bacterial growth was followed by qPCR. Analyses by SEM allowed studying the iron and chlorine absorption by fungi, and finally, *Fourier Transform Infrared* (FTIR) and *Raman Spectroscopies* were used to identify the bio-minerals produced.

#### Conclusions

The results show that microbes have the potential to stabilize iron objects by both reduction into more stable minerals and by removing chloride from the object.



## **FEMS-2105**

### **Microbial transformations of metals and metalloids**

#### **USE OF ENDOSPORE-FORMING FIRMICUTES FOR THE BIOREMEDIATION OF TRACE-METALS**

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<sup>1</sup>*Microbiology, University of Neuchâtel, Neuchâtel, Switzerland*

#### **Background**

In urban environments, run-off water constitutes a sink for metals as it collects dust particles produced by human activities. In the case of the city of Lausanne (Switzerland), run-off and wastewater is not yet entirely separated. As a consequence, the wastewater treatment plant of Lausanne rejects metal contaminated effluent water into the lake of Geneva, leading to an enrichment of lake sediment by metals like copper, cadmium and zinc. In this environment a link between metal contamination and the relative abundance of endospore-forming Firmicutes was clearly observed. Further investigations tend to show that bacterial exudates, massively produced by these bacteria, play an important role in the chelation and immobilization of metals.

#### **Objectives**

The objective of this study is to achieve a better understanding of this relation between endospore-forming Firmicutes and metals in aquatic environments. Is it particular to environments under the influence of wastewater treatment plant rejections or could it be generalized to other polluted environments? The chelation mechanism is also of particular interest.

#### **Methods**

To achieve this, sediment cores from different location (two different lakes) were analyzed, looking for bacterial communities composition (454-pyrosequencing), but also physico-chemical characteristics, like metals content (ICP-MS measurement). Cultivation experiments in media amended with different concentrations of metals was also initiated, first for evaluating the chelation potential of the different strains tested (ICP-OES), second for isolating and investigating the chelation substances that were produced (SEM and FTIR).

#### **Conclusions**

A better understanding of the metal immobilization processes by bacterial biomass could lead to promising bioremediation technologies.

**FEMS-0906**

**Archaea and life in extreme environments**

**The archaellum: How archaea swim**

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Motility is an important feature for all life forms. Whereas bacteria use the well studied flagellum for swimming motility, archaea employ the archaellum, which is a rotating type IV pilus like structure. The crenarchaeal archaellum is only composed of seven subunits that are essential for the assembly and rotation of the archaellum. Next to the structural filament protein FlaB, the *S. acidocaldarius* archaellum operon encodes for the monotopic membrane proteins FlaX, FlaF and FlaG, whereas the motor complex is formed by the nucleotide binding protein FlaH, the ATPase FlaI and the polytopic membrane protein FlaJ.

Our current functional and structural analysis of the different archaellum components will be discussed as well as their role in assembly and rotation of the archaellum.

## FEMS-2834

### Archaea and life in extreme environments

#### Archaea in Cryoenvironments

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This presentation will describe recent highlights of our research examining polar microbial biodiversity and microbial ecology in extreme polar cryoenvironments with a focus on the Archaeal components. In general, we have detected archaeal sequences (haloarchaea, methanogens, *Crenarchaeota*, *Thaumarchaeota*) from 16S surveys and metagenome sequencing projects in most of the habitats tested including Arctic and Antarctic permafrost soils, gypsum and sandstone cryptoendoliths, and saline and hypersaline cold perennial springs in the high Arctic of Canada, albeit at relatively less proportions (10 fold or greater) than Bacteria sequences. The Lost Hammer (LH) spring in the Canadian high Arctic perennially discharges subzero (-5°C) hypersaline (~24% salt) brines through thick layers of permafrost (> 500 m), and so far accounts for the only described terrestrial methane seep in frozen settings on Earth and serves as an analogue for possible Martian liquid water habitats. In the LH source sediments, archaeal phylotypes related to signatures from hypersaline deep-sea methane-seep sediments and were dominated by the anaerobic methane group 1a (ANME-1a) clade of anaerobic methane oxidizing archaea while spring channel sediments was dominated by phylotypes most closely related to ammonia-oxidizing *Thaumarchaeota*. Viral morphologies (fusiform, spherical) characteristic of archaeal viruses are present in the cold saline springs. Haloarchaea and methanogens are readily found in most permafrost soils and their role in CH<sub>4</sub> fluxes from melting Arctic permafrost environments will be presented. Overall, the roles and activities of *Archaea* in cryoenvironments, especially under ambient subzero conditions, remains very poorly elucidated and is significantly hampered by the lack of culturable archaeal representatives capable of subzero growth.

**FEMS-1447**

**Archaea and life in extreme environments**

**MOLECULAR MECHANISMS OF ACTION BY A TETR-FAMILY TRANSCRIPTION FACTOR IN SULFOLOBUS ACIDOCALDARIUS**

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**Background**

Archaea, however also found in mainstream habitats, are characterized by several extremophilic species. Extremophilic microorganisms have more robust proteins and unique metabolic strategies to survive extreme conditions. *Sulfolobus acidocaldarius*, an aerobic thermoacidophilic crenarchaeon, is a promising host for biotechnological processes.

**Objectives**

Getting insight in the mode of action of transcriptional mechanisms could lead to a better understanding of archaeal physiology and to an extended transcriptional toolbox for genetic engineering of *Sulfolobus*. Gene regulation in response to environmental conditions is crucial for the fitness and survival.

This project focuses on the characterization of a TetR-like transcription factor, SaFadR.

**Methods**

A variety of biochemical techniques and *in vivo* experiments has been implemented to study DNA-binding behaviour and physiological role.

**Conclusions**

By analysing the interactions of the SaFadR protein with genomic regions, SaFadR binds *in vivo* ten genomic regions, with the highest affinity to its own promoter region and the one of a juxtaposed, divergently transcribed operon. Other ChIP-seq binding peaks were observed for an operon encoding enzymes involved in the  $\beta$ -oxidation pathway. This binding is confirmed *in vitro* by performing EMSA. Addition of a fatty acyl-CoA reduces the *in vitro* binding.

Using a SaFadR knockout strain, the relative expression levels of SaFadR and the other possible target genes were compared with an isogenic wild type by qRT-PCR.

Based on these results we can conclude that the SaFadR protein is a fatty acid responsive transcription factor that represses genes involved in fatty acid and lipid metabolism in a fatty acyl-CoA dependent manner.



**FEMS-1783**

**Archaea and life in extreme environments**

**POTENTIAL PH ADAPTATION MECHANISMS IN THE NOVEL ACIDOPHILIC IRON OXIDISER “FERROVUM” SP. JA12 INVOLVE A UREASE**

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**Background**

Members of the novel proposed order “*Ferrovales*” are the first acidophilic iron oxidising bacteria within the *Betaproteobacteria*.

**Objectives**

Since neutrophiles and acidophiles seem to use different strategies to maintain pH homeostasis and to cope with acid stress we were interested in the strategies of the novel acidophile “*Ferrovum*” sp. JA12.

**Methods**

Therefore we sequenced and assembled the complete genome sequence of “*Ferrovum*” sp. JA12, derived from the acid mine drainage of a lignite mining site.

**Conclusions**

The genome analysis revealed the gene repertoire for both the typical strategies of acidophiles and of neutrophiles. Like other acidophiles “*Ferrovum*” sp. JA12 may be able to create a reverse membrane potential by the increased uptake of potassium ions facilitated by a number of predicted potassium transporters. In “*Ferrovum*” sp. JA12 cytoplasmic buffering may be accomplished by amino acid decarboxylases or the production of polyphosphates and spermidine. The presence of a urease encoding gene cluster was unexpected since there are so far no similar gene clusters in other acidophilic iron oxidisers. “*Ferrovum*” sp. JA12 may use the urease to create a cytoplasmic buffering system similar to the pathogenic neutrophile *Helicobacter pylori*. Furthermore, the genome contains numerous genes encoding chaperones and DNA repair systems to cope with damages to proteins and DNA caused by low pH. Taken together “*Ferrovum*” sp. JA12 uses strategies to inhibit uncontrolled influx of protons, to cope with excess protons and to deal with cellular damages in order to maintain life in acidic environments.

## FEMS-2099

### Archaea and life in extreme environments

## SYSTEMS BIOLOGY X-TREME: METABOLISM AND ITS CHALLENGES AT HIGH TEMPERATURE

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### Background

**Background.** Archaea are characterized by a complex metabolism with many unique enzymes that differ from their bacterial and eukaryotic counterparts. *Sulfolobus solfataricus* (optimal growth at 80°C, pH 3) is known for its metabolic versatility and is able to utilize a great variety of different carbon sources (e.g. polymers, pentoses, sugar acids, aldehydes). However, the underlying degradation pathways and their regulation are largely unknown.

### Objectives

**Objectives.** In the SulfoSYS<sup>BIOTEC</sup> project we analyse growth on different carbon sources using an integrated Systems Biology approach. Besides pathway elucidation metabolic constraints for life at high temperature due to metabolite instability are of special interest.

### Methods

**Methods.** Systems Biology approaches (genomics, transcriptomics, proteomics, metabolomics, bioinformatics, modelling, classical microbiology, biochemistry as well as genetic/ molecular biology techniques).

### Conclusions

**Conclusions.** Using a combined modelling and *in vitro* reconstruction approach it could be shown that in *S. solfataricus* the thermal instability of triosephosphates leads to carbon loss and futile cycling. Like in the modified branched Entner-Doudoroff pathway for D-glucose degradation also in the newly discovered pathway for L-fucose the formation of triosephosphates is omitted ((i) L-fucose oxidation to L-fuconate via a promiscuous dehydrogenase, (ii) dehydration to 2-keto-3-deoxy-L-fuconate via dehydratase, (iii) 2-keto-3-deoxy-L-fuconate cleavage to pyruvate and L-lactaldehyde via aldolase and finally (iv) L-lactaldehyde conversion to L-lactate via aldehyde dehydrogenase). This pathway shows interesting overlaps to the D-arabinose pathway representing another example for pathway promiscuity in *Sulfolobus* species. Thus, circumventing triosephosphate formation appears to be a special metabolic

thermoadaptation strategy in *Sulfolobus* to face the special challenge of metabolism at high temperature.



**FEMS-1327**

**Archaea and life in extreme environments**

**PURPLE PHOTOSYNTHETIC REACTION CENTERS FOUND IN THE RARE BACTERIAL PHYLUM GEMMATIMONADETES**

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**Background**

Photosynthetic bacteria represent one of the earliest organisms on Earth. Despite over three billion years of evolution, organisms containing (bacterio)chlorophyll based reaction centers have so far been reported in only six out of over thirty bacterial phyla.

**Objectives**

In search for novel photosynthetic species we conducted a large screening of microorganisms inhabiting several extreme aquatic habitats.

**Methods**

One of the surveyed lakes was the Swan lake in the western Gobi Desert, Inner Mongolia, China. The screening was performed using a custom made infra-red screening system.

**Conclusions**

Using this approach we isolated a novel red pigmented strain AP64 belonging to the poorly characterized bacterial phylum Gemmatimonadetes. The new organism contains fully functional type-2 (pheophytin-quinone) photosynthetic reaction centers composed of bacteriochlorophyll *a* and carotenoids of spirilloxanthin series. Full genome sequencing revealed the presence of a 42.3-kb long photosynthesis gene cluster (PGC) in its genome. This makes Gemmatimonadetes the seventh bacterial phylum containing chlorophototrophic species (after Cyanobacteria, Proteobacteria, Chlorobi, Chloroflexi, Firmicutes and Acidobacteria). The organization and phylogeny of its photosynthesis genes suggested their ancient acquisition via horizontal transfer from purple phototrophic bacteria. It challenges our understanding of the evolution of photosynthesis providing the first evidence that photosynthetic capacity can be transferred between distant bacterial phyla.

For more info: <http://www.pnas.org/content/111/21/7795.abstract>

**FEMS-2870**

**Toxin-antitoxin systems in bacteria: clinical implications**

**Role of TA modules in *Salmonella* persists during infection**

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Bacterial persisters are generally non-growing cells that are tolerant to antibiotic treatment. They have been studied mainly in *Escherichia coli in vitro* and are reported to form stochastically at very low frequency. All bacterial species form persisters and they are particularly relevant in the context of chronic infections that are recalcitrant to antibiotic treatment. *Salmonella enterica* causes acute and chronic infections by replicating and surviving for long periods of time within host cells, notably macrophages. Previously we described a dual fluorescence reporter method, Fluorescence Dilution, which enables heterogeneity of intracellular bacterial replication to be followed at the single cell level. Using this method we found many viable non-replicating bacteria in several organs in the mouse model of typhoid caused by *Salmonella*. We provide evidence that interaction with the host triggers formation of intracellular persisters that are likely to constitute a reservoir for relapse of infection by *Salmonella* and we decipher the host triggering signals and the bacterial actors involved in this phenomenon.

**FEMS-2816**

## **Toxin-antitoxin systems in bacteria: clinical implications**

### **Type II toxin-antitoxin systems: a reservoir for more sophisticated functions?**

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### **Background**

Type II toxin-antitoxin systems (TAs) are composed of two genes that encode a toxic protein and its cognate antitoxin. TAs are surprisingly abundant in bacterial and archeal genomes. The reason of this evolutionary success remains largely unknown. Such 'toxin-antitoxin-like' functions are also found in complex secretion systems targeting bacterial or eukaryotic cells and relying on secreted toxins.

### **Objectives**

Our hypothesis is that type II toxic domains are recruited by genetic systems encoding sophisticated functions such as type III, IV and type VI secretion systems as well as bacterial contact-dependent growth inhibition system (CDI and Rhs).

### **Methods**

Using bioinformatics approaches, a series of domain of unknown function (DUF) was detected in bacterial genomes and predicted to be *bona fide* toxins belonging to TAs and to be associated with CDI and/or Rhs or to be homologous to effectors of type III secretion systems. Functionality of putative toxic domains and antitoxin/immunity is tested in *E. coli*. Toxin activity and targets are investigated.

### **Conclusions**

Our data show that domains from TAs or CDI/Rhs systems or homologous to type III effectors are toxic for *E. coli* and that toxicity is rescued by co-expression of the antitoxin/immunity cognate protein. These domains were shown to inhibit translation, although through different means, suggesting that new targets might be possibly involved.

In conclusion, recruitment of toxic domains encoded by TAs by secretion systems might constitute a strong selective pressure. This may provide an explanation why TAs are evolutionary successful and widespread in bacterial genomes.

**FEMS-0881**

**Toxin-antitoxin systems in bacteria: clinical implications**

**DISTINCT TYPE I AND TYPE II TOXIN-ANTITOXIN MODULES CONTROL  
SALMONELLA LIFESTYLE INSIDE EUKARYOTIC CELLS**

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**Background**

*Salmonella enterica* serovar Typhimurium (S. Typhimurium) is a Gram-negative bacteria which can infect and proliferate within several eukaryotic cells lines. This pathogen can persist in the infected organisms during a prolonged period of time and is responsible for human diseases ranging from gastroenteritis to systemic disease. This species, as most bacteria, possesses Toxin-Antitoxin Systems (TAS) in its genome. TAS are operons composed of two genes: an unstable antitoxin and a stable toxin, which can be activated in response to different stress conditions. The toxins targets are important for cell proliferation or viability. In this way, TAS can regulate bacterial cell growth and can induce biofilm formation, persistence, and probably dormancy during infections.

**Objectives**

To characterize TAS of S. Typhimurium and measure their role in infection.

**Methods**

TAS search was done using different data bases. Three different cell lines (two types of fibroblasts and HeLa cells) were selected for infection assays. Intracellular TAS expression was monitored by proteomics and RT-qPCR.

**Conclusions**

We report a systematic survey and functional analysis of TAS found in S. Typhimurium. Our analyses showed that 18 out of 27 identified TAS of S. Typhimurium are *bona fide* systems. Although we could detect expression of 10 TAS under eukaryotic cell infection, only 5 systems (3 type I and 2 type II modules) contributed to intracellular survival.

**FEMS-0739**

**Toxin-antitoxin systems in bacteria: clinical implications**

**FIC DOMAIN TOXINS ARE THE ORIGIN OF INTRA- AND INTER-KINGDOM EFFECTORS OF BARTONELLA**

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**Background**

The diverse and abundant proteins containing FIC (filamentation induced by cAMP) domains typically perform AMPylation, the transfer of an adenosine-5' monophosphate onto target proteins. Research has so far focused on scattered representatives that secondarily evolved into bacterial virulence factors, but their original functions and evolutionary trajectories have remained elusive.

**Objectives**

We studied the molecular function and biological role of bacterial FIC domain proteins that are related to host-targeted effector proteins of *Bartonella* in order to understand their evolution.

**Methods**

We used protein biochemistry and proteomics to identify the molecular function of bacterial FIC domain proteins. Classical microbiology and advanced agarose gel electrophoresis were used to study the effects of these proteins inside bacterial cells, particularly on DNA topology.

**Conclusions**

One major group of bacterial FIC domain proteins are toxins of a novel toxin-antitoxin (TA) module that acts via the AMPylation of DNA gyrase and topoisomerase IV, the two bacterial type IIA topoisomerases. AMPylation inhibits all activities of the targets and thus causes a disruption of cellular DNA topology that induces bacterial persistence, a dormant state of multidrug tolerance. One of these FIC domain toxins, VbhT, is encoded next to a type IV secretion system (T4SS) in *Bartonella* and unique

in that it contains a cognate secretion signal. This protein and the associated T4SS are closely related to host-targeted effectors of this widespread mammal pathogen and their secreting machinery. We therefore propose that VbhT is an interbacterial effector protein and constitutes a “missing link” in the evolution of host-targeted effectors from genuine bacterial proteins.

**FEMS-1190**

**Toxin-antitoxin systems in bacteria: clinical implications**

**RESPONSIVE DIVERSIFICATION LEADS TO ANTIBACTERIAL TOLERANCE  
THROUGH ACTIVATION OF A PORE-FORMING TOXIN**

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**Background**

Within a bacterial population, a small fraction of persister cells is transiently capable of surviving exposure to lethal doses of antibiotics. As a bet-hedging strategy, persistence levels are determined both by stochastic induction and by environmental stimuli, called responsive diversification.

**Objectives**

Our results support a central role for the conserved GTPase Obg in determining persistence in *Escherichia coli* in response to nutrient starvation. In this work we unravel the underlying molecular mechanism.

**Methods**

We used single cell methods to correlate Obg levels with persistence and identified the stringent response as a contributing pathway by using knockout strains. Furthermore, we performed a transcriptome study to identify downstream genetic elements. This resulted in the identification of HokB, a pore-forming toxin and part of a toxin/antitoxin module, as an effector of Obg-mediated persistence. To assess the role of HokB in this phenomenon, we showed membrane depolarization upon *obg* and *hokB* overexpression. Furthermore, repolarization of the bacterial membrane by means of a light-driven proton pump diminishes the effect of Obg and HokB on persistence, demonstrating a causal link between the membrane potential and the persistence phenotype.

**Conclusions**

We propose a model for Obg-mediated persistence that requires the stringent response alarmone (p)ppGpp and proceeds through transcriptional control of the *hokB-sokB* type I toxin-antitoxin module. In individual cells, increased Obg levels induce HokB expression, which in turn results in a collapse of the membrane

potential, leading to dormancy. Combined, our findings signify an important step towards unravelling genetic mechanisms underlying persistence.



**FEMS-1957**

**Toxin-antitoxin systems in bacteria: clinical implications**

**MOLECULAR BASES OF THE ALLOSTERIC SWITCH IN CONDITIONAL COOPERATIVITY REGULATION OF TOXIN-ANTITOXIN OPERONS**

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**Background**

Conditional cooperativity is the paradigm of transcription regulation of type II toxin-antitoxin (TA) operons and is intricately related to persistence. Conditional cooperativity allows the toxin component of toxin-antitoxin modules to act as a co-repressor at low toxin:antitoxin ratios and become an activator at high toxin:antitoxin ratios. A common feature in the majority of antitoxins is the presence of an intrinsically disordered region (IDR) typically involved in toxin neutralization and repressor complex formation.

**Objectives**

To address how the antitoxin IDR is involved in transcription regulation, using the *phd/doc* operon as model system.

**Methods**

We determined crystal structures of Phd in complex with its operator box. The DNA-binding domain of Phd interacts with DNA in a novel fashion. Moreover thermodynamic and structural characterization of Phd in complex with the operator region of the operon in different repression states reveals that a phenotypic switch from negative to positive cooperativity, involving the IDR, is the core of the regulatory process.

**Conclusions**

In absence of Doc, the intrinsically disordered part of the repressor bound to the DNA acts as a "veil" covering the second site and precluding the binding of a second Phd molecule, resulting in strong negative cooperativity and weak repression. The presence of Doc drives the folding upon binding of the additional Phd molecule with the consequent increase in affinity and strengthening repression, up to the point when the excess of Doc overwhelms the system leading to its de-repression. Our model has important implications in the regulation of bacterial operons involving intrinsically disordered transcription factors.

**FEMS-2836**

**Fungal bacterial interactions**

**Microbial logistics - Mycelia as networks for functional transport of bacteria and chemicals**

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Biodegradation of contaminants in soil is a logistic problem. 'Logistics'; refers to have 'the right thing, at the right place, at the right time'. Degrading bacterial communities walk the tightrope of contaminant availability and suitable physical habitats for their metabolic activity; this situation is often aggravated by the concurrence of restricted bacterial mobility and retarded transfer of hydrophobic organic contaminants (HOC). In order to cope with heterogeneous soil environments mycelial fungi therefore have developed a unique network-based growth form. Unlike bacteria mycelia spread efficiently in the soil, penetrate air-water interfaces and cross over air-filled pores between the bacteria and contaminants in the vadose. In air-filled soil, enhanced homogenization of bacteria and contaminants can be achieved by bridging physical air gaps with fungal hyphae thus enabling substrate-directed mobilization of bacteria along chemical gradients. In this contribution we demonstrate the biodegradation-enhancing influence of mycelial dispersal networks on both the microbial transport and the translocation of HOC. Here we show that mycelial networks (i) act as effective dispersal networks for both undirected and targeted mobilization of contaminant degrading bacteria ('fungal highways'), (ii) act as hotspots for horizontal gene transfer, (iii) increase the mobility of a wide range of HOC due to their translocation in their cytoplasmic streaming ('fungal pipelines'), and (iv) improve the accessibility of bacteria to soil contaminants and their subsequent biodegradation. Given their ubiquity and length of up to  $1000 \text{ m g}^{-1} \text{ dry soil}$  mycelia may play a significant role for the ecosystem service of contaminant biodegradation. Knowledge of 'logistic fungus bacteria interactions' may also be exploited in biotechnological approaches beyond soil remediation, such as in food industry or the production of biofuels.

**FEMS-2349**

**Fungal bacterial interactions**

**Fungi-bacteria interactions: from soil functioning to complex behavior**

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Although the role of fungi and bacteria in processes such as decaying or nutrient cycling in soils has been established, the part played by the interactions between these microbial taxa in soil functioning is still poorly known. Myriads of potential interactions can emerge as results of the encounter of fungi and bacteria in a shared environment. These interactions range from positive (e.g. mutualism) to negative (e.g. antagonism). I will present results for several of these types of interactions including fungus-driven bacterial dispersal, bacterial farming by fungi, as well as biocontrol and niche exclusion between bacteria and fungi. For those, we have started to understand the underlying principles that have allowed the emergence and maintenance of these interactions within an evolutionary conceptual frame. In addition, we are investigating the molecular and chemical mechanisms that regulate processes such as partner recognition or metabolic activity. All of these elements are addressed in the context of the importance of fungi-bacteria interactions in ecosystem functioning and services, in particular related to pedogenic carbon storage.

In the past few years, the scientific community has started to recognize the importance of fungi-bacteria interactions in fields as varied as human health (e.g. infection and obesity), agriculture (e.g. plant growth promotion), or the environment (e.g. carbon storage and nutrient cycling). In the future it will be important to compare the mechanisms discovered in the case of soil fungi-bacteria interactions with other ecosystems, in order to understand the universality of the processes that allow these phenomena to emerge in nature.

**FEMS-1314**

**Fungal bacterial interactions**

**EFFECT OF DISPERSAL NETWORKS ON BACTERIAL DISPERSAL AND BIODEGRADATION AT VARYING WATER POTENTIALS**

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**Background**

In porous media the matric and the osmotic potential contribute to the availability of water to microbes and decisively influence important microbial ecosystem services such as biodegradation. Bacterial motility is considered as a key driver for biodegradation and fungal mycelia have been shown to serve as effective dispersal networks thereby increasing bacterial movement in water unsaturated environments.

**Objectives**

However, poor knowledge exists on the beneficial effects of mycelia at varying water potentials ( $\Psi_w$ ).

**Methods**

We therefore established experimental microcosms to investigate the effect of mycelia-like dispersal networks on the dispersal and growth of *Pseudomonas putida* KT2440-gfp at given osmotic and matric potentials and determined their benefit for the biodegradation of benzoate. Using either NaCl or polyethylene glycol 8000 the  $\Psi_w$  of agar was modified between  $\Delta\Psi_w$  0 - -1.5 MPa (i.e. water potentials representing completely saturated or plant permanent wilting point conditions).

**Conclusions**

We found that dispersal, growth and biodegradation rates dropped noticeably below  $\Delta\Psi_w$  -0.5 MPa in osmotically stressed systems. However, in matric stress treatments this decline occurred at  $\Delta\Psi_w$  -0.25 MPa due to a complete repression of bacterial movement at this  $\Psi_w$ . The presence of dispersal networks effectively defused the negative effects of lowered matric potentials by enhancing bacterial dispersal. No beneficial network effect was observed in the osmotically stressed systems, likely due to NaCl toxicity rather than the water deprivation effects. We propose that dispersal networks act as an important buffer mechanism and hence may increase the microbial ecosystem's functional resistance to matric stress.

**FEMS-2291**

**Fungal bacterial interactions**

## **STRATEGIES OF STREPTOMYCETES FOR FUNGAL TARGETING AND INHIBITION**

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### **Background**

Streptomyces belong to actinobacteria, have a complex life cycle, and comprise many species. They have a large linear chromosomal DNA, which has been sequenced from many species. They are highly abundant in different soil-types and the rhizosphere of plants. They can play a role in association with selected organisms, and sometimes as symbionts. The knowledge about proteins and compounds facilitating these interactions is still scarce.

### **Objectives**

The aim of the presented investigations was to investigate the role of newly discovered proteins and vesicles during the interaction of streptomyces with selected fungi.

### **Methods**

Characterization of proteins and metabolites using LC-MS, electron-microscopy, fluorescence microscopy, cloning of genes, and analyses of designed mutants.

### **Conclusions**

Streptomyces produce several unique proteins that target selected fungi provoking close interaction. We purified the proteins and elucidated their mode of action.

In addition, we discovered firstly that several proteins and metabolites of streptomyces reside in vesicles, which we characterised by several biochemical and microscopic tools. We succeeded to monitor the in vivo generation of vesicles and to propose of a model for their biogenesis. The application of vesicles to selected fungi including a plant pathogen, led to pronounced fungal damages. The investigations revealed the sophisticated *Streptomyces* repertoire to target and to suppress fungi

including a plant pathogen.

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**FEMS-2618**

**Fungal bacterial interactions**

**ROLE OF VOLATILES IN ANTIFUNGAL ACTIVITY OF A LACTOBACILLUS  
PARACASEI AGAINST PENICILLIUM STRAINS**

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**Background**

Fungi constitute a major spoilage problem in many foods including dairy products. The use of specific lactic acid bacteria (LAB) with antifungal properties has shown potential for delaying fungal spoilage without compromising sensory acceptability of the products. Much effort has been put into isolating and identifying antifungal metabolites from active fractions of cell free ferments of these bacteria. However, the methods used have been suboptimal for detection of volatiles.

**Objectives**

To study the antifungal role of volatiles.

**Methods**

Inhibitory effect of a *Lactobacillus paracasei* and metabolites was studied in a newly developed chemically defined medium and yogurt against two spoilage-associated *Penicillium* strains using a gentle sample treatment.

**Conclusions**

Diacetyl was identified as the major volatile produced. Antifungal activity was observed in both solid and liquid media as well as in yogurt. If cells were removed from the ferment both diacetyl content and antifungal activity was drastically reduced. If formation of diacetyl was inhibited by enzymatic conversion of the pre-cursor antifungal activity decreased markedly underlining the importance of volatile compounds in the inhibitory activity.

**FEMS-1851**

## **Fungal bacterial interactions**

### **THE 'FUNGAL HIGHWAY' TOLL: METABOLISMS AND GENES INVOLVED IN FUNGAL-DRIVEN BACTERIAL DISPERSAL IN NATURAL ECOSYSTEMS**

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<sup>4</sup>Biogeosciences laboratory, University of Lausanne, Lausanne, Switzerland

#### **Background**

Bacterial motility in water-unsaturated soil is usually highly restricted. Previous experiments *in vitro* however have demonstrated that bacteria may take the 'fungal highway', i.e. are able to actively move along fungal hyphae.

#### **Objectives**

In order to get better insights into such fungal-bacterial interactions *in situ*, we developed a column system designed to collect and cultivate fungi and associated migrator bacteria at varying growth conditions.

#### **Methods**

Our column system was placed into a soil known to exhibit high metabolic fungal-bacterial interactions within the oxalate-carbonate pathway. After 4 to 8 days, the columns were removed, bacteria and fungi that grew in the columns identified, and the bacteria analyzed for their ability to use different carbon sources, fix nitrogen and to encode the type-III secretion system (as possible indicator for migrating activity along hyphae).

#### **Conclusions**

We identified *Fusarium* sp. and *Chaetomium* sp. as fungi, and *Achromobacter* sp., *Acinetobacter calcoaceticus*, *Ochrobactrum* sp., *Olivibacter* sp., *Pseudomonas* sp., *Stenotrophomonas* sp. and *Variovorax* sp. as associated migrator bacteria. We found that the ability of bacteria to move along their fungal hosts was medium-dependent. Most of the bacteria were able to fix dinitrogen. This points to a possible trophic exchange between bacteria and fungi. Except for one bacterial strain genes encoding type-III secretion systems were not found in the isolated bacteria.

Our findings propose that fungal hyphae are not an inert path taken by motile bacteria; 'fungal highways' interactions seem to be mediated by trophic exchanges between fungi and bacteria. Therefore, migrator bacteria selection might be based on their metabolic capabilities.



**FEMS-0593**

**Natural products including secondary metabolites**

**Metabolically talented bacteria from microbial dark matter**

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Environmental studies suggest that a large fraction of the total bacterial diversity has so far resisted cultivation. Many of the uncultivated bacteria belong to large taxonomic groups without cultivated representatives and known functional properties. Considering the importance of bacteria for drug discovery, it has been proposed that these elusive organisms represent a massive untapped resource of novel compounds and biotechnologically useful enzymes. To investigate the biosynthetic potential of microbial dark matter, our laboratory uses metagenomic and single-cell methods focusing on host-associated microbiomes. We provide evidence for the existence of a new environmental candidate phylum, termed "Tectomicrobia", members of which live associated with marine sponges and are metabolically highly versatile. These bacteria exhibit a rich specialized metabolism with bioactive compounds not yet encountered in cultivated microbes. Functional studies revealed unprecedented types of biosynthetic transformations that are of significant biotechnological interest.

## **FEMS-1924**

### **Natural products including secondary metabolites**

#### **High-throughput chemotyping and phenotyping using *Pseudomonas aeruginosa* deletion libraries**

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We are currently profiling two *P. aeruginosa* single-gene deletion libraries (in the clinically relevant strains, PA01 and PA14) in a large number (>200) of chemical and environmental stresses. In these assays, we obtain quantitative measurements both for the fitness, the ability of each mutant to form biofilms and their secondary metabolite profile.

The quantitative phenotypic and chemotypic signature for each mutant will be later used to a) identify novel gene function; b) map metabolic pathways c) map interconnections between functional processes; d) address the role of horizontal gene transfer in network rewiring and gene repurposing; d) understand how chemical perturbations effect the secondary metabolite profile; e) identify novel secondary metabolites; etc.

At the workshop I will present the basis of our methodology, a progress report on the ongoing screen and proof-of-principle examples highlighting the different types of information we are obtaining. Another aspect of the talk will be how to handle and mine such a diverse data set (<16k MS&MS2 profiles, <12800k independent phenotypic data points).

**FEMS-0761**

**Natural products including secondary metabolites**

**METABOLOMIC PROFILING AND GENOMIC STUDY OF A MARINE SPONGE-ASSOCIATED STREPTOMYCES SP.**

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<sup>3</sup>*School of Biomedical Sciences, Curtin University, Perth, Australia*

<sup>4</sup>*Department of Microbiology, University College Cork, Cork, Ireland*

**Background**

The increase in antibiotic resistance among clinical microbes has created a need to develop antimicrobials with novel modes of action.

**Objectives**

As part of a marine biodiscovery programme, sponge-associated microbes were screened to identify novel metabolites.

**Methods**

Metabolomics and genomics are two complementary platforms for analysing an organism as they provide information on the phenotype and genotype, respectively. These two techniques were applied in the dereplication and identification of bioactive compounds from a *Streptomyces* sp. (SM8) isolated from the sponge *Haliclona simulans* from Irish waters. Three distinct bioactives that include an antibacterial fatty acid derivative, and separate antifungal and anti-calcineurin activities were identified. Antimycin compounds were identified in the antifungal fractions. Genomic analysis revealed the presence of multiple secondary metabolism gene clusters, including a cluster for the antimycin family of compounds. When the extract of the mutant strain was analysed, it showed loss of antimycin production and significantly less antifungal activity than the wild-type strain. Butenolides were identified as compounds showing anti-calcineurin activity<sup>1</sup>.

**Conclusions**

The combined metabolomic and genomic approach used in this study resulted in the identification of antimycins, polyhydroxylated saturated fatty acids and butenolides as

major antifungal and antibacterial and anti-calcineurin metabolites of SM8, respectively. The genome sequence provides a genetic framework to study their biosynthesis and their potential role in regulation of gene expression.

#### References:

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**FEMS-1175**

**Natural products including secondary metabolites**

## **MOLECULAR GENETICS OF SIMOCYCLINONES BIOSYNTHESIS: SURPRISING ASSEMBLY OF THE TETRAENE CHAIN**

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### **Background**

Simocyclinone D8 and its' derivatives were isolated from *Streptomyces antibioticus* Tü6040. They exhibit antibiotic activity against Gram-positive and Gram-negative bacteria, and are highly potent inhibitors of DNA gyrase supercoiling with an IC<sub>50</sub> lower than that of novobiocin<sup>1,2</sup>.

### **Objectives**

However, biosynthetic studies and bioengineering of simocyclinones were limited because of difficulties in genetic manipulations of the producer strain. We have discovered the new strain *Kitasatospora* sp. producing novel simocyclinone.

### **Methods**

*Kitasatospora* sp. genome was sequenced and detailed *smc* cluster annotation has been performed as well as its alignment to published *sim* gene cluster from *S. antibioticus* Tü6040<sup>3</sup>. Inactivation and heterologous expression of several biosynthetic genes have been performed to prove their function.

### **Conclusions**

Several significant differences in the architecture of the new *smc* gene cluster have been found in comparison to the previously published *sim* gene cluster from *S. antibioticus* Tü6040. Most striking, genes responsible for the tetraene chain formation (type I PKS in *sim* cluster) could not be detected. Instead, three genes with the high similarity to ACP and two beta-ketoacyl-synthases were annotated within the *smc* cluster (*ORF2*, *ORF3* and *ORF4*, respectively).  $\Delta$ *ORF4* mutant was deficient in production of the D-type simocyclinones, only angucyclines with the attached sugar could be detected. Subsequent complementation of the mutant with the intact *ORF4* restored biosynthesis of simocyclinones. Furthermore, inactivation of the KS domen in type I PKS from *sim* cluster in *S. antibioticus* Tü6040 didn't influence biosynthesis of the simocyclinone D8. Obtained data suggest new unusual pathway for linear polyketide assembly during simocyclinone biosynthesis.



## FEMS-1996

### Natural products including secondary metabolites

#### HETEROLOGOUS PRODUCTION OF ANTIBACTERIAL SECONDARY METABOLITES FROM *SERRATIA MARCESCENS* IN *PSEUDOMONAS PUTIDA*

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#### Background

Microorganisms produce a huge variety of secondary metabolites that often exhibit several valuable bioactivities such as antibiosis. This wealth is often accessed by heterologous biosynthesis in a host microbe. Several challenges are typically encountered here, concerning i) the establishment of the genetic information underlying a biosynthetic pathway, ii) the complex biosynthesis and iii) the host's tolerance towards antibiotic products.

#### Objectives

*Pseudomonas putida* is an outstanding host candidate for the heterologous production of substances with antimicrobial activity. The strain *P. putida* KT2440 is a well-established "safety strain" which is amenable to genetic modification, possesses a versatile metabolic background and withstands many antimicrobial compounds with high tolerance.

Here, we aimed to produce the secondary metabolites prodigiosin and serrawettin W1 from *Serratia marcescens*. Besides other highly valuable properties such as anticancer activity, both the tripyrrolic red pigment prodigiosin and the lipopeptide serrawettin W1 exhibit antibacterial activity, rendering their heterologous production challenging.

#### Methods

For heterologous production of prodigiosin, the entire prodigiosin gene cluster was inserted into the chromosome of *P. putida*. For the production of serrawettin W1, a single synthase gene was expressed from a plasmid in the host. Products were isolated and antimicrobial activities were verified.

## Conclusions

We successfully established production of the two antibacterial natural products prodigiosin and serrawettin W1 in the bacterial host *P. putida*, thereby enabling further studies on their bioactivities. This study further highlights that *P. putida* is highly suited for the expression of complex secondary metabolite pathways and the efficient production of antimicrobial compounds.



**FEMS-2617**

**Natural products including secondary metabolites**

**TILIVALLINE, SECONDARY METABOLITE OF INTESTINAL PATHOBIONT  
KLEBSIELLA OXYTOCA INHIBITS EPITHELIAL CELL PROLIFERATION**

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**Background**

*Klebsiella oxytoca* is a natural resident of the human gut microbiota. During antibiotic-induced intestinal dysbiosis colonic overgrowth of this bacterium rapidly leads to antibiotic-associated hemorrhagic colitis (AAHC). We have shown that bacterial production of the secondary metabolite tilivalline, which is structurally related to pyrrolobenzodiazepines, induces apoptosis of epithelial cells, disrupts their barrier function, and is key to the pathogenesis of colitis in an animal model of AAHC (1). The impact of tilivalline on host physiology in healthy carriers of low (commensal) levels of *Klebsiella oxytoca* is unknown.

**Objectives**

We synthesized tilivalline chemically to investigate the human cellular processes altered by this enterotoxin in vitro.

**Methods**

HeLa cells were treated with a range of tilivalline concentrations and cellular stress responses were monitored on the transcriptional level using qRT-PCR, the protein level via Western analysis, through microscopic imaging and FACS as well as by biochemical analysis.

**Conclusions**

Apoptosis is induced by high doses of tilivalline. Low concentrations of the cytotoxin inhibit HeLa cell proliferation. The position of cell cycle arrest was defined via quantification of cyclin transcripts, DNA damage response markers, modulation of checkpoint pathways, analysis of spindle apparatus formation and centrosome dynamics.

(1) Schneditz, G., Rentner, J., Roier, S., Pletz, J., Herzog, K.A.T., Bückner, R., Troeger, H., Schild, S., Weber, H., Breinbauer, R., Gorkiewicz, G., Högenauer, C., Zechner, E.L. (2014) PNAS 111, 13181-13186

## **FEMS-3093**

### **New diagnostic approaches**

#### **Rapid antimicrobial susceptibility testing : will it be genotypic or phenotypic?**

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#### **Rapid antimicrobial susceptibility testing: will it be genotypic or phenotypic?**

Clinicians are used to get sequential results from the microbiology lab.

For blood cultures, it is classical to report as soon as possible the presence of growth and the morphology of the bacteria that are observed under the microscope (e.g. Gram-negative rods), then an identification (e.g. *E. coli*) and finally the antimicrobial susceptibility profile, AST (e.g. amoxillin R, co-amoxicillin S, etc...). The clinician learns therefore several clinically-relevant elements, on a sequential manner, typically over a few days: i) there is bacterial growth in this sample, ii) the identification of the bacteria may suggest a particular anatomic source (e.g. likely from urinary or digestive origin), and finally, iii) the AST permits reducing the antibiotic spectrum.

MALDI-TOF MS has allowed reducing the time for bacterial identification, but this is typically insufficient to permit targeted antimicrobial therapy. There is therefore a major incentive in knowing as soon as possible how to better target antimicrobial therapy.

Sequencing (and especially with the availability of bench-top NGS systems) can provide an extensive listing of DNA sequences that are present in a sample. Irrespective of the numerous technical challenges (sample preparation, DNA extraction/purification, DNA sequencing, data analysis with or without assembly, etc...), sequence interpretation remains a major hurdle, and can also fail to capture bacterial properties affecting the AST (e.g. the activity of an extrusion pump, ...). Clinically-relevant diagnostic devices may therefore need -at least in the initial phase- to be coupled with some advanced phenotypic information of diverse nature (peptides, mRNA, enzymatic activity, etc...).

This strategy and possible implementation will be discussed during the lecture.

## **FEMS-2619**

### **New diagnostic approaches**

#### **Whole genome sequencing as a tool for clinical diagnostics**

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The introduction of Whole Genome Sequencing (WGS) through the Next Generation Sequencing (NGS) technology has great potential as a typing as well as a diagnostic tool in the clinical laboratory. In principal, WGS data contain all relevant information for typing and diagnostic analysis. Existing gene based (local) typing and diagnostic data are often easily extracted from WGS data and many studies have shown the potential of WGS data for (global) analysis of clonal relationship in outbreaks. However, a prerequisite for the transition from current typing and diagnostic methods to methods based on WGS data is to identify the added value of this transition such as increased speed of analysis, decreased costs as well as cheap (free) and user-friendly harmonised WGS-based tools. Also, it will to some extent require the establishment of backward compatibility with existing typing methods, which are currently well-established tools for both clinical and epidemiological investigations. In addition to this, great efforts in relation to standardization and validation of WGS tools as well as harmonization of methods will be a requirement for a successful transition. Finally, WGS has the potential to detect and possibly type pathogens directly in complex samples (the so-called *metagenomics*), rather than analysing and comparing single isolates. The benefits from this include decreased time for diagnostics and the ability to detect pathogens, which are not easily detected by standard laboratory methods. However, tools and methods to analyse metagenome data is still in their infancy and will require further maturation before implementation directly in a clinical setting.

**FEMS-0870**

**New diagnostic approaches**

**AN INTEGRATED ANALYSIS WITH DEEP-SEQUENCING OF BACTERIAL INFECTION AND PROTEOMIC PROFILING OF NEUTROPHILS IMPROVES PREDICTION OF CLINICAL OUTCOMES IN SEPSIS**

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**Background**

Sepsis is a life-threatening complication caused by a serious microbial infection. Due to the complexity of the sepsis response, the prognosis of sepsis is poor and outcomes in individual patients are difficult to predict.

**Objectives**

This study aims to define molecular signatures of bacterial infection at various stages of sepsis progression and associate them with the clinical features and outcomes of sepsis patients.

**Methods**

Two thousand three hundred forty-three patients who underwent elective general surgery were recruited prospectively at Jinling Hospital in China from June 2013 to September 2014. A set of 180 patients had six groups that reflected conventional concepts of sepsis progression as a pyramid. Molecular patterns of bacterial infection were elucidated by 16S rDNA-based high-throughput sequencing techniques. We also performed a comparative proteomic profiling on neutrophils derived from peripheral blood of the patients.

**Conclusions**

The bacterial profiles of patients who were diagnosed as sepsis and ultimately died within 28 days differed markedly from those of survivors with sepsis. The different taxa of bacterial profiling clustered into the following genus: *Escherichia/Shigella*, *Stenotrophomonas*, *Acinetobacter*, *Pseudomonas*, *Corynebacterium* and *Propionibacterium*. A total of 97 proteins were characterized as differentially expressed between nonsurvivors and sepsis survivors, indicating impaired functions of neutrophils in lysosome, chemotaxis, transmigration, phagocytosis and intracellular killing of microorganisms. Our data demonstrate that an in-depth analysis of peripheral blood bacteria with measurements of six protein molecules on neutrophils

would predict survival of sepsis patient. This algorithm may help to guide the treatment of individual patients with sepsis.

**FEMS-1611**

**New diagnostic approaches**

**CAMPYLOBACTER DETECTION IN POULTRY MEAT BY USING AN ORGANIC LIGHT-EMITTING DIODE BASED BIOCHIP.**

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**Background**

Nine million cases of campylobacteriosis, due to handling and/or consumption of undercooked poultry meat contaminated with *Campylobacter*, occur each year in Europe, with a cost to public health systems of about EUR 2.4 billions. The direct plating and/or enrichment culture methodologies based on the ISO 10272-1B: 2006 are the methods typically used, but the long time required by the classical methods to grow microorganisms, can lead to the distribution of contaminated food. Molecular biology with PCR, qPCR and blotting techniques have greatly improved the techniques by reducing the time required to obtain results, but integrated biochips are the ideal solution for producing portable diagnostic systems.

**Objectives**

The aim of the work was the development of a miniaturised, highly-sensitive DNA biochip based on a deep-blue organic light-emitting diode (OLED) to analyze real poultry samples.

**Methods**

Two specific DNA probes (capture and detection) were designed and used to analyze real poultry meat samples. The capture probe was bonded to a glass slide while the detection probe was labelled with a Alexa Fluor fluorophore to be detected by the utilization of the OLED.

**Conclusions**

The results obtained by the biochip were compared with PCR, broth enrichment and colony growth methods, to demonstrate the relevance of this system. The OLED biochip reached a sensitivity of 0.37 ng/μL DNA, which was approximately 20-fold higher than the sensitivity obtained with dot blot assay (25 ng/μL) and didn't need an enrichment step. This system exploits a multi-disciplinary approach, and shows high sensitivity, quick test results and ease of use.

**FEMS-1438**

**New diagnostic approaches**

**MOLECULAR TYPING OF TREPONEMA PALLIDUM ISOLATED FROM PATIENTS ATTENDING THE UNIVERSITY HOSPITAL OF BUENOS AIRES, ARGENTINA**

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**Background**

*Treponema pallidum* subsp. *pallidum*, the causative agent of syphilis, is a sexually-transmitted disease with worldwide occurrence. Molecular typing of *T. pallidum* provides valuable epidemiological information, including determination of macrolide resistance.

**Objectives**

This study was aimed to type, for the first time in South America, *T. pallidum* isolates in clinical samples

**Methods**

During 2013, 79 samples were collected from patients with clinical signs of syphilis: 72 lesion swabs, 3 whole blood samples and 4 cerebrospinal fluid. DNA was extracted and PCR amplification of *poIA*(TP0105), *tmpC*(TP0319), TP0136, TP0548, and 23S rRNA was performed(1). A CDC typing scheme was made including the determination of 60-bp repeats in the *arp* gene, RFLP of *tprE*-G-J and sequence analysis of TP0548(2). The human MTHFR gene was amplified to test for PCR inhibitors.

**Conclusions**

Treponemal DNA was detected in 57.3%(43/75) of samples (four were excluded). A total of 36 samples (83.7%) were typed. Nine genotypes were detected using the sequencing-based typing, with SSS being the most frequent. CDC typing scheme

was done in 22 samples, and 7 genotypes were detected, with 14d/d being the most common. In 13.9% of the samples, mutations causing macrolide resistance were detected. In conclusion, during this first molecular typing study, low frequency of macrolide resistant mutations was detected compared to Europe and the US. Although some detected treponemal genotypes were identical to those described in other countries, several genotypes were new, not found outside Argentina.

**References:**

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- 2- J Infect Dis. 2010 Nov 1;202(9):1380-8.



## FEMS-2303

### New diagnostic approaches

#### RAPID AND SENSITIVE ISOTHERMAL DNA AMPLIFICATION ON A DIGITAL MICROFLUIDIC DEVICE FOR THE DETECTION OF ANTIBIOTIC RESISTANT BACTERIA

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#### Background

Antibiotic resistance is a growing global threat to human health. There is a clear need to develop rapid and sensitive tests to determine antibiotic resistance as part of diagnosis so that antibiotics can be prescribed to treat infections in a targeted manner. The most rapid, sensitive and specific molecular assays are based on nucleic acid amplification to detect specific genes of interest. Combining this with droplet microfluidic platforms represents the most promising approach for portable point-of-care assays.

#### Objectives

To develop a miniaturised assay to detect the gene encoding for a CTX-M enzyme, an extended spectrum  $\beta$ -lactamase present in common bacteria such as *E. coli*.

#### Methods

We perform real time isothermal DNA amplification by recombinase polymerase amplification (RPA) on a digital microfluidic device, which utilises active matrix electrowetting on dielectric (AM-EWOD) technology.

#### Conclusions

The assay can detect just 10 copies of the *E. coli* CTX-M gene within 15 minutes. Continuous mixing during the assay run on AM-EWOD enhances the sensitivity of the assay by 10-fold relative to a commercial microplate reader. The assay is quantitative, with the time of onset of amplification relating to the logarithm of DNA copies in a linear manner. DNA from *E. coli* containing the CTX-M gene can be detected even when it is mixed 1:100 with genomic DNA from *E. coli* without CTX-M. Our systems ability to rapidly detect antimicrobial resistance genes represents a promising step to a complete and fully automated, sample in – answer out, Point of Care diagnostic assay suitable for directing antibiotic prescribing.

**FEMS-1223**

**White biotechnology**

### **Protein Engineering and Enzyme Cascade Reactions**

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Protein engineering has developed in the past decade to a highly important technology (1,2) to create enzymes with desired properties, but also to understand their function. Whereas initially rational protein design based on detailed analysis of structures was the method of choice, directed evolution became an important alternative. In this lecture, the principle strategies and current challenges in protein engineering will be highlighted. We used information from protein databases to create 'small, but smart' focused protein libraries of enzymes from the a/b-hydrolase fold family using the 3DM database (3). This resulted in enzyme variants with enhanced thermostability or enantioselectivity (4). For the synthesis of chiral amines, we developed an *in silico* analysis and identified a toolbox of novel (*R*)-selective transaminases (5) as well as (*S*)-selective enzymes from a structure-guided search (6). More recently, we focused on cascade reactions to convert unsaturated fatty acids to  $\omega$ -hydrocarboxylic acids (7), or to combine alcohol dehydrogenase, enoate reductase and monooxygenase for *in vivo* biotransformations (8). In another project, we use a chalcone isomerase in the conversion of flavonoids (9). Very recently, we developed a synthesis of  $\epsilon$ -caprolactone-oligomers through an enzyme cascade (10).

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**FEMS-2794**

**White biotechnology**

**Discovery and engineering of enzyme systems for the transformation and degradation of explosives**

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**Background.** The contamination of the environment with toxic explosives presents a serious and widespread problem at sites across the world. Our recent studies on the biodegradation of energetic compounds have resulted in the isolation of bacteria from explosive-contaminated land that are capable of metabolizing 2,4,6-trinitrotoluene (TNT) and RDX. Our work has targeted the use of genetic engineering for broadening and improving the degradative capabilities of biological systems for the remediation of explosives pollution. Significant progress has been made towards this goal and we have successfully combined the biodegradative capabilities of soil bacteria with the high biomass and stability inherent to plants. In an effort to determine how plant tolerance could be further improved, we have been investigating the enzymology underlying the innate ability of plants to detoxify explosives.

**Objectives.** 1. To determine the biochemistry and molecular genetics of explosives metabolism in microorganisms and plants. 2. To characterise the enzymes mediating these degradative pathways. 3. To engineer transgenic plant systems for the remediation of explosives contaminated land.

**Methods.** The work has required a plethora of modern molecular techniques and spans methods in microbial and plant biology through to methods in synthetic biology and enzyme engineering.

**Conclusions.** These studies have identified a number of interesting enzymes including a unique cytochrome P450 XplA from a strain of *Rhodococcus rhodochrous* that degrades RDX. We have successfully engineered transgenic plants that can efficiently remove toxic levels of TNT and RDX from contaminated soil and water. As a result of recent advances in our knowledge of the biodegradation of explosives, endogenous plant detoxification systems and the use of genetic engineering, our work on transgenic plants is now poised for testing in the field.

**FEMS-1860**

**White biotechnology**

## **FERMENTATION OF CRUDE SYNGAS FROM THE BIOLIQ® PLANT KARLSRUHE**

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### **Background**

The new bioliq® pilot plant at the KIT covers the complete process chain required for producing customized fuels from dry lignocellulosic biomass. For energy densification of the biomass, fast pyrolysis is applied. The liquid pyrolysis oil and solid char obtained can be processed further in the entrained flow-gasifier to tar-free, low-methane raw synthesis gas. Prior to chemically catalysed fuel synthesis a multistep cleaning of raw synthesis gases is performed: Particles, alkaline salts, HCl, H<sub>2</sub>S, COS, CS<sub>2</sub>, NH<sub>3</sub>, and HCN are removed to avoid catalyst poisoning during fuel synthesis. The pilot plant is equipped with an innovative hot-gas cleaning system.

### **Objectives**

Acetogenic bacteria are able to ferment syngas to a variety of organic acids and alcohols. In contrast to the catalysts used in the Fischer-Tropsch process, these biological catalysts can process a broad range of syngas compositions and deal with impurities like sulphur compounds or CO<sub>2</sub>. At the moment intense efforts are made to genetically modify *C. ljungdahlii* to optimize product yields and establish synthesis routes to new products. To assess industrial large scale applicability of these strains it will be necessary to determine their performances with crude syngas, as each purifying step will decrease the economy of the process.

### **Methods**

Our aim is to establish a platform process for evaluating the use of different qualities of raw syngas obtained from the different steps of Karlsruhe bioliq® plant.

### **Conclusions**

Therefore, a setup of multiple 2 L bioreactors with online gas and product analysis is currently developed in our lab

**FEMS-1857**

**White biotechnology**

**HIGH LEVEL PRODUCTION OF ETHYLMALONYL-COA PATHWAY-DERIVED DICARBOXYLIC ACIDS BY METHYLOBACTERIUM EXTORQUENS UNDER COBALT-DEFICIENT CONDITIONS AND BY POLYHYDROXYBUTYRATE NEGATIVE STRAINS**

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**Background**

There is a high need for sustainably producible chemical building blocks being applicable e.g. as monomers for novel bioplastics. The ethylmalonyl-CoA pathway (EMCP) harbors several CoA-esters such as ethylmalonyl-, methylsuccinyl- or mesaconyl-CoA whose free dicarboxylic acid derivatives potentially present promising synthons for chemical industry. The EMCP in *Methylobacterium extorquens* offers the possibility to produce these new building blocks directly from the cheap and non-food competing C-source methanol.

**Objectives**

Main objective of this work was the optimization of dicarboxylic acid production with *M. extorquens* strains expressing a thioesterase towards an industrially relevant process.

**Methods**

Suitable thioesterase enzymes were identified by *in vitro* assays and the product spectra were analyzed by *in vivo* experiments. Expression of thioesterases in different *M. extorquens* strains was increased by optimization of ribosome binding sites. To increase carbon flux into product and avoid acid reuptake we used metabolic engineering and medium optimization strategies. First high cell density fermentations were performed in a bioreactor.

**Conclusions**

Suitable thioesterases for production of different EMCP-derived dicarboxylic acids were identified and their expression levels in *M. extorquens* were optimized. Productivities of different strains were compared and increased by different metabolic engineering and medium optimization strategies. In summary, we present the biotechnological production of unconventional dicarboxylic acids from methanol with high efficiency.

**FEMS-2742**

**White biotechnology**

## **PLANT SECONDARY METABOLITE PRODUCTION IN SACCHAROMYCES CEREVISIAE**

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### **Background**

The diversity of metabolites of plants with an estimated 200,000 different compounds is unrivaled by any other group of organisms. Many of these metabolites have special features, ranging from scents to antimicrobial agents. The limited availability of secondary metabolites often hinders their exploitation. This can be overcome by implementing synthesis pathways into the yeast *Saccharomyces cerevisiae* that has an excellent track record in white biotechnology.

### **Objectives**

We constructed and characterized *S. cerevisiae* microbial cell factories producing triterpenoids.

### **Methods**

Advanced metabolic engineering including compartment engineering was employed to redistribute carbon flux through the mevalonate pathway and squalene synthesis towards triterpenoids. The product performance of the engineered strains was investigated by bioprocess engineering, while advanced analytics delivered targets for the next round of strain improvement.

### **Conclusions**

*S. cerevisiae* was engineered for triterpenoid production. In fermentations considerable titers of these valuable secondary plant metabolites could be produced. The results will be presented in the context of the required molecular tools and novel engineering targets.

**FEMS-2450**

**White biotechnology**

### **POLYMER BIOTECHNOLOGY: BIOMIMETIC CONCEPTS**

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### **Background**

In nature microbes are equipped with enzymes and helper proteins to modify and degrade naturally occurring polymers including polyesters. Due to huge environmental problems, the development of efficient polyester recycling strategies and improvement of biodegradable polyesters that show required material properties is necessary. Learning strategies from microbes enables us to fight against these ecological problems and process polymers efficiently.

### **Objectives**

Here, strategies based on novel microbial enzymes for tailor-made polyester modification and degradation are presented. Mimicking nature, enzymes have been genetically equipped with polymer binding modules [1]. Moreover, mechanistic insights into the enzymatic hydrolysis of synthetic polyesters like polyethylene terephthalate and the compostable synthetic polyester poly(butylene adipate-co-butylene terephthalate) by cutinases and novel “anaerobic” esterases are given.

### **Methods**

The enzymatic hydrolysis of polyesters by cutinases e.g. from *Thermobifida cellulosilytica* (Thc\_Cut1) and novel anaerobic esterases from different *Clostridium* spp. was investigated via HPLC-MS. Additionally, fusion proteins of Thc\_Cut1 and binding modules of polyhydroxyalkanoate depolymerases and combination with hydrophobins from *Trichoderma* spp. [2] were investigated.

### **Conclusions**

Novel enzymes isolated from anaerobic microorganisms were demonstrated to degrade synthetic polyesters. The adsorption as well as the activity of microbial



polyesterases was increased when fused to different binding modules or when hydrophobins were applied when compared to the native enzyme.

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**FEMS-2838**

**Microbial symbioses with marine animals**

**How to eat without a mouth or gut: symbioses between chemosynthetic bacteria and gutless marine worms**

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Marine worms without a mouth or gut were first discovered over 35 years ago in coral reef sediments, but the mystery of how they gain their nutrition was not solved until large communities of gutless invertebrates were discovered in 1977 at hydrothermal vents in the deep sea. We now know that these animals gain their nutrition from chemosynthetic bacteria that fix carbon dioxide into organic compounds, as in photosynthesis, but using reduced compounds such as sulfide or methane as energy sources instead of sunlight. Chemosynthetic symbioses occur worldwide and have evolved multiple times from numerous bacterial lineages and at least 9 animal groups (Dubilier et al. 2008. *Nature Rev Microbiol*). In my talk, I will present an overview of the research in my lab on these symbioses and describe how new tools that range from in situ instruments for measuring environmental parameters to lab-based methods such as 'omics' and single cell imaging, provide the opportunity to understand symbiotic associations in their environmental and ecological context.

**FEMS-2867**

**Food biotechnology**

**The transcriptome landscape of *Lactococcus lactis*: regulatory RNAs uncovered**

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The lactic acid bacterium *Lactococcus lactis* is of high importance in biotechnology, specifically in the dairy industry. It is used in starter cultures world-wide for the production of safe and nutritious milk fermentation products. Rapid acidification of milk through lactose utilization and proteolytic activity are key parameters in determining product quality. The large-scale industrial processes in which these bacteria are employed put them under a variety of intrinsic and extrinsic (technological) stresses. Consequently, stress response systems in *L. lactis* have been extensively studied, as have industrially important global transcriptional regulators of carbon and nitrogen metabolism.

It is becoming increasingly clear that, in addition to protein regulators, small non-coding RNAs play important regulatory roles in bacteria. Recent developments in next-generation RNA sequencing and in bioinformatics have revolutionized transcriptome analyses. As a result, novel non-coding RNAs have been discovered in many micro-organisms and subsequent studies have uncovered several regulatory mechanisms that operate at the level of RNA.

The presence and roles of non-coding RNAs with regulatory functions have not yet been assessed in *L. lactis*. Because of their presumed importance, we set out to identify novel RNA elements, such as small regulatory RNAs, antisense RNAs and sORFs, by growing the organism under various (stressful) conditions. Using differential RNA sequencing, a transcriptome landscape of *L. lactis* was obtained that was automatically mined using TSSer and verified and refined manually. Thus, we identified hundreds of novel RNA species. Experimental validation was performed for a selection of these potential RNA regulators. The role of one non-coding RNA located in the 3'UTR of the gene for the regulator of e.g. the *arc*-operon in *L. lactis*, ArgR, will be further detailed.

## FEMS-2815

### Food biotechnology

#### Understanding the mechanisms of yeast adaptation to wine fermentations

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The wine fermentation is a complex process produced as a result of the activities of a succession of microorganisms. Although *S. cerevisiae* is the most frequent species in wines, and the subject of most studies, *S. bayanus* var. *uvareum* and natural hybrids between *Saccharomyces* species such as *S. cerevisiae* x *S. kudriavzevii* and *S. cerevisiae* x *S. bayanus* var. *uvareum* are also involved in wine fermentations and can be preponderant in certain wine regions. Studies performed in our laboratory, showed that strains of non-conventional *Saccharomyces* species, such as *S. bayanus* var. *uvareum* and *S. kudriavzevii*, exhibit physiological properties of potential interest in enology because they can respond to the new demands of the wine industry, such as their ability to ferment at low temperatures, their increased **production of glycerol**, their lower ethanol yield (1, 2, 3). The main objective of this work is to decipher the molecular mechanisms responsables of the overproduction of glycerol in *S. kudriavzevii*, *S. bayanus* var. *uvareum* and natural strains of *S. cerevisiae* (4, 5), as well as the molecular basis involved in the cold adaptation of *S. kudriavzevii* strains (6).

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**FEMS-0527**

**Small RNAs and regulation: basic aspects Offered by FEMS Microbiology Reviews**

**Hfq- and RNA-mediated regulation in *Pseudomonas aeruginosa* metabolism and pathogenicity**

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**Background**

The RNA chaperone Hfq has been recognized as the principle post-transcriptional regulator of carbon catabolite repression (CCR) in the opportunistic human pathogen *P. aeruginosa* (PAO1). Hfq was shown to act as a translational repressor that prevents ribosome loading through binding to A-rich sequences within the ribosome binding site of mRNAs encoding catabolic enzymes. Furthermore, the non-coding CrcZ RNA was shown to bind to and to sequester Hfq, which in turn abrogates Hfq-mediated translational repression.

**Objectives**

To study the role of *P. aeruginosa* Hfq and of the regulatory RNA CrcZ in anoxic biofilm formation and antibiotic susceptibility.

**Methods**

Biochemical and genetic methods as well as next generation sequencing were employed to address the impact of Hfq and CrcZ on anoxic biofilm formation and antibiotic susceptibility of *P. aeruginosa*.

**Conclusions**

Comparative transcriptome studies identified CrcZ as being up-regulated in anoxic biofilms of PA14 grown under conditions that mimic growth in the cystic fibrosis lung. We show that CrcZ up-regulation suppresses biofilm formation, which most likely results from sequestration of Hfq. Moreover, RNA-seq analyses revealed functions that account for decreased biofilm formation upon CrcZ up-regulation. In addition, we show that CrcZ affects the susceptibility of PA14 towards several antibiotics, which in turn can explain the differential susceptibility of *P. aeruginosa* to certain antibiotics during planktonic and biofilm growth, respectively.

**FEMS-0664**

**Small RNAs and regulation: basic aspects Offered by FEMS Microbiology Reviews**

**Small regulatory RNAs in type I toxin-antitoxin systems**

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Toxin-antitoxin (TA) systems are pairs of adjacent genes in which a stable toxic peptide is neutralized by an unstable antitoxin. A broad variety of type I TA systems are known in which the antitoxin is a small RNA that is convergently transcribed to the toxin mRNA, and inhibition occurs by RNA/RNA binding. The antitoxin either inhibits translation or facilitates degradation of the toxin mRNA. We investigate two type I systems from the *B. subtilis* chromosome, *bsrG*/SR4 and *bsrE*/SR5. The toxins are small hydrophobic peptides, and toxin and antitoxin genes overlap at their 3' ends by ≈120 nt. Whereas BsrG (38 aa) causes cell lysis in the absence of its antitoxin SR4, BsrE (30 aa) is only toxic upon overexpression. The antitoxins SR4 and SR5 promote toxin mRNA degradation by recruiting RNase III. In contrast to SR5, SR4 has a second function: it induces structural changes around the *bsrG* SD sequence, thus further obstructing ribosome binding which results in translational inhibition.

Secondary structures of toxin RNA, antitoxin and their complexes were mapped, apparent binding rate constants ( $k_{app}$ ) of wild-type and mutated toxin/antitoxin pairs determined, and the results corroborated by *in vivo* experiments. The binding pathways of toxin mRNA and antitoxin were elucidated by employing time-course experiments. In both cases, binding initiates between the antitoxin terminator-loop and loop 4 of toxin mRNA.

RNases involved in the degradation of toxin mRNAs and antitoxins were identified. RNase R plays the major role in *bsrG*/SR4, whereas RNase J1 is a crucial player in *bsrE*/SR5. Antitoxin-induced RNase III cleavage sites in *bsrG* and *bsrE* mRNAs were mapped.

Whereas *bsrG*/SR4 is the first temperature-sensitive TA system, factors affecting *bsrE*/SR4 expression have still to be identified.

**FEMS-2697**

**Small RNAs and regulation: basic aspects Offered by FEMS Microbiology Reviews**

**New players in the field of small regulatory RNAs**

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Bacterial small RNAs (sRNAs) are crucial regulators of cellular functions by modulating gene expression in response to various environmental changes. Many bacterial species were shown to express hundreds of sRNAs. These sRNAs regulate target mRNAs by direct base-pairing to positively or negatively affect their translation and stability. In *E. coli* and other Enterobacteriaceae, the sRNA RyhB regulates iron homeostasis. Under iron-rich conditions, the transcriptional repressor Fur blocks *ryhB* transcription. During conditions of iron starvation however, Fur becomes inactive and relieves repression of *ryhB*. Under these conditions, RyhB directly regulates approximately 20 different mRNAs encoding iron-using proteins. By binding to those mRNAs, RyhB shuts down translation and stimulate their rapid degradation through the action of the endoribonuclease E (RNase E). RyhB contributes actively to increase the levels of free intracellular Fe<sup>2+</sup> (iron sparing) under conditions of iron starvation by reducing the expression of iron-using proteins and by stimulating siderophore synthesis. Surprisingly, although we have used microarrays to successfully determine the effect of RyhB on the transcriptome of *E. coli*, this approach was insufficient to determine the regulation of undetected but important mRNAs. Thus sRNAs can significantly modulate many target mRNAs without interfering with their levels, which unfortunately prevents target detection by classical techniques. To address this challenge, we developed a method based on affinity purification and RNA sequencing, which could identify any RNA molecule interacting with sRNAs, regardless of the regulation. Thus, we used sRNAs tagged with the bacteriophage MS2 RNA stemloops, which are bound with high specificity by the MS2 coat protein. We then analysed all bound target RNAs by RNA sequencing. We will present new targets that suggest an unexpected but important mechanism for bacterial cells.

**FEMS-2381**

**Small RNAs and regulation: basic aspects Offered by FEMS Microbiology Reviews**

**Regulatory RNAs in the pathogenic Epsilonproteobacteria, *Helicobacter pylori* and *Campylobacter jejuni***

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Genome sequencing revealed the potential proteins and a high genetic diversity of *Helicobacter pylori*, the causative agent of gastritis and gastric cancer, and the related, emerging food-borne pathogen *Campylobacter jejuni*. So far, little is known about post-transcriptional regulation in these pathogenic Epsilonproteobacteria. However, based on a differential RNA-sequencing approach (dRNA-seq), we had defined a genome-wide map of transcriptional start sites (TSS) and identified >60 small RNAs (sRNAs) in *H. pylori*. To understand how transcriptome differences could contribute to phenotypic differences among strains, we applied a comparative dRNA-seq approach to multiple *C. jejuni* strains. Our study revealed that the majority of TSS is conserved among strains, but we also observed strain-specific promoter usage and sRNA repertoires, which could contribute to strain-specific gene regulation. Based on our transcriptome datasets, we are now using *Helicobacter* and *Campylobacter* as new model organisms for riboregulation in virulent bacteria and bacteria without the RNA chaperone Hfq. We are functionally characterizing abundant sRNAs and are especially interested in their roles and underlying molecular mechanisms in stress response and virulence control. For example, we could show that the highly abundant and conserved sRNA, RepG, from *Helicobacter* directly base-pairs with a homopolymeric G-repeat in the mRNA leader of the chemotaxis receptor TlpB and that length variation of this G-repeat determines the outcome (repression or activation) of RepG-mediated post-transcriptional regulation. More recently, we have identified additional RepG targets, including genes involved in LPS biosynthesis or oxidative stress response, and a potential functional RepG homolog in *C. jejuni*. The example of RepG shows that identifying and studying sRNAs in bacteria without Hfq can reveal new twists in RNA-mediated regulation and will provide new insights into regulatory mechanisms and virulence control of bacterial pathogens.



## **FEMS-3149**

### **Anaerobic physiology and biochemistry Offered by FEMS Letters**

#### **Energy Metabolism of Sulfate Reducing Prokaryotes**

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The dissimilation of sulfur compounds is likely to have been one of the first energy metabolisms on the early Earth [1]. However, many questions remain about how sulfur-metabolizing organisms obtain energy for growth from reducing, oxidizing or disproportionating sulfur compounds. In the case of sulfate reduction, the terminal reductases involved (APS reductase, AprAB, and dissimilatory sulfite reductase, DsrAB) have long been identified, but how these two reactions are coupled to energy conservation is still not clear. DsrAB, in particular, is a key enzyme in dissimilatory sulfur metabolism, being present not only sulfate/thiosulfate/sulfite reducing organisms, but also in sulfur-oxidizers and sulfur disproportionators.

Our lab has studied several of the key proteins involved in sulfate reduction, including AprAB, DsrAB and two respiratory membrane complexes specific to sulfur-metabolizing organisms, QmoABC and DsrMKJOP, which are involved in the electron transfer pathways with AprAB and DsrAB [2]. These complexes hint at the possibility of menaquinone involvement and chemiosmotic energy conservation, during sulfate reduction. In addition, a genomic analysis of energy metabolism genes in sulfate reducers suggested that the recently recognized process of electron bifurcation may also be involved [3], and that there are conspicuous links between sulfate reducers and methanogenic organisms [1].

In this talk I will present an overview of the role of these proteins in sulfate reduction, with a special focus on the function of the small protein DsrC as a physiological partner of DsrAB and the DsrMKJOP complex [4].

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**FEMS-2294**

**Anaerobic physiology and biochemistry Offered by FEMS Letters**

**New Insights into the Metabolism of Nitrate-driven Anaerobic Oxidation of Methane**

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Methane oxidation is an important process to prevent emission of the greenhouse gas methane and further exacerbating of climate forcing. Both aerobic and anaerobic microorganisms have been reported to catalyze methane oxidation with only few substrates as electron acceptors. New microorganisms were discovered that coupled the oxidation of methane to nitrate or nitrite reduction [1-3].

Here, we investigated such an enrichment culture on physiological, biochemical, transcriptomic and genomic level to establish a metabolic model of nitrate-driven anaerobic oxidation of methane (nitrate-AOM). Nitrate-AOM is catalyzed by an archaeon closely related to methanogens. Methane may be activated by methyl-CoM reductase and subsequently undergo full oxidation to carbon dioxide via reverse methanogenesis. All enzymes of this pathway were present and expressed in the investigated culture. The genome of the archaeal culture encoded a variety of proteins involved in a membrane-bound electron transport chain similar to those found in *Methanosarcina* species. Nitrate reduction seems to be located in the extracellular space and may be catalyzed by Nxr- or Nar-like protein complexes. The enrichment culture produced mainly nitrite but also some ammonium during nitrate reduction, presumably by the action of an extracellular Nrf enzyme. One of the key questions is how electrons from cytoplasmic reverse methanogenesis reach the nitrate reduction enzymes in the extracellular space. We will present a tentative model, based on genome and transcriptome studies, how electrons from reverse methanogenesis are transported to the nitrate and nitrite reductases, thereby suggesting how central energy metabolism of nitrate-driven anaerobic oxidation of methane could work.

This work was supported by the ERC AG 339880 and the SIAM Gravitation Grant 24002002.

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**FEMS-1652**

**Anaerobic physiology and biochemistry Offered by FEMS Letters**

**Microbial life under extreme energy limitations: how to make a living by producing acetate from hydrogen and carbon dioxide**

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Acetogens constitute an important limb in anaerobic food webs. Synthesis of acetate from carbon dioxide and molecular hydrogen *via* the Wood-Ljungdahl pathway is thermodynamically at the edge of life since it allows or the synthesis of only a fraction of an ATP. How the pathway is coupled to the net synthesis of ATP has been an enigma for a long time, but recently, new insights have been obtained using the anaerobic, acetogenic bacterium *Acetobacterium woodii* as a model system (1,2). The energy conserving module comprises of a Na<sup>+</sup> translocating ferredoxin-NAD oxidoreductase (Rnf complex) (3) and an unusual Na<sup>+</sup>-F<sub>1</sub>F<sub>0</sub> ATP synthase (4). Endergonic reduction of ferredoxin with H<sub>2</sub> as electron donor is achieved by energy coupling *via* electron bifurcation (5). Reduction of CO<sub>2</sub> to formate with NADH would be highly endergonic and this energetic barrier is overcome by a novel hydrogen-dependent CO<sub>2</sub> reductase (6).

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## **FEMS-2649**

### **Anaerobic physiology and biochemistry Offered by FEMS Letters**

#### **Metal reduction in bacteria**

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Under anaerobic conditions many microorganisms are able to utilize insoluble metal species during respiration. In Gram-negative organisms this requires electrons that accumulate in the menaquinol pool to be transported across the periplasm, through the outer membrane and ultimately transferred to an extracellular electron acceptor. One of the best-studied organisms capable of metal respiration is the facultative anaerobe *Shewanella oneidensis*, which produces a range of multiheme cytochromes that facilitate electron transfer to extracellular minerals.

During mineral respiration *S. oneidensis* utilizes a porin-cytochrome complex to shuttle electrons from the periplasm across the outer membrane. This complex is comprised of a periplasmic decaheme cytochrome (MtrA), a 28-strand porin (MtrB) and an outer membrane decaheme cytochrome (MtrC) that forms the interface between the cell and mineral surfaces. During anaerobic respiration *S. oneidensis* also secretes trace amounts of flavin mononucleotide (FMN) and this has been linked to effective reduction of insoluble metals. FMN was originally proposed to function as a soluble electron shuttle mediating electron exchange between extracellular cytochromes and the mineral surface, however we have recently shown that FMN binds tightly to the extracellular MtrC, becoming a eleventh cofactor under anaerobic conditions. These findings, coupled with new structural and kinetic information have allowed us to reconsider how the cytochromes of *Shewanella* are used during dissimilatory metal reduction.

**FEMS-2797**

**Cell-cell communication / Quorum sensing: basic aspects**

**Unravelling autoinducing peptide biosynthesis and recognition in staphylococci**

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The *agr* quorum sensing (QS) system plays a pivotal role in the co-ordination of virulence in *Staphylococcus aureus* and other closely related Gram-positive bacteria. The *agr* locus consists of two divergent transcriptional units controlled by the *agrP2* and *agrP3* promoters. The *agrP2* operon consists of four genes, *agrBDCA*. AgrB processes the pre-propeptide AgrD to generate a macrocyclic auto-inducing peptide (AIP). This is exported from the cell and subsequently recognized by AgrC, a transmembrane receptor kinase that activates the response regulator, AgrA. This drives expression of the *agrP2* operon completing the autoinduction loop as well as the *agrP3* operon which codes for both delta-toxin and a regulatory RNA, RNAIII, the primary effector of the *agr* response. The *agr* locus has undergone evolutionary divergence such that the AIPs of four different *S. aureus agr* groups self-activate but cross-inhibit. Consequently to retain functionality, any changes in the AIP-encoding gene (*agrD*) that results in modification of the AIP structure must be accompanied by corresponding changes in the AgrC receptor. To better understand the biosynthesis and recognition of staphylococcal AIPs we have exploited both *in vivo* and *in vitro* chemical biology approaches to gain new insights into the function of AgrB and the structural features of the AIPs and AgrC that determine activation or inhibition of the sensor kinase. This work is of particular relevance to the design of *agr* inhibitors as antivirulence agents for the prevention and treatment of staphylococcal infections.

**FEMS-2358**

**Cell-cell communication / Quorum sensing: basic aspects**

**Interspecies and interkingdom signaling in plant associated bacteria**

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Just like what occurs in humans, plants have been recently recognized as meta-organisms possessing a distinct microbiome which has close relationship with their associated microorganisms. The plant microbiome presents an additional reservoir of genes that the plant can have access to when needed. Plant health is thought to heavily depend also on its microbiome and plants most probably intimately affect bacterial gene expression via a communication highway of signals produced by bacteria and plants. These complex highways will become clearer through the detection of signal molecules and identifying the effects on gene expression of microbial and plant exudates. Although plant microbiome composition is now being well studied, there is hardly any information on these signals.

Interspecies and interkingdom signaling, is now a fast developing field of research; we are using plant associated bacteria to investigate these types of communication. We have identified a new sub-family of bacterial LuxR proteins which is widely present in plant associated proteobacteria which binds and consequently responds to plant signals. In addition we are using a plant disease as model of interspecies bacterial interactions which also highlights the role of these interactions in bacterial plant pathogenicity. Understanding these types of communication between microbiomes and plants are a step in the right direction for disease management.

## FEMS-0615

### Cell-cell communication / Quorum sensing: basic aspects

#### The DSF family of bacterial cell-cell signal molecules

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The Diffusible Signal Factor (DSF) family of cell-to-cell signalling molecules comprises *cis*-2-unsaturated fatty acids that differ in their chain length and branching. DSF signalling was first described in the plant pathogen *Xanthomonas campestris* (Xcc) where the signal was identified as *cis*-11-methyl-dodecenoic acid. The synthesis and perception of the DSF signal in Xcc require products of the *rpf* gene cluster (for regulation of pathogenicity factors). The synthesis of DSF is dependent on RpfF, whereas the RpfC/RpfG two-component system is implicated in DSF perception and signal transduction. RpfC is a complex hybrid sensor kinase whereas the RpfG regulator has a CheY-like receiver domain attached to an HD-GYP domain, which acts to degrade the second messenger cyclic di-GMP, leading to regulation of the expression of virulence factors. Recent work has described a second receptor for DSF in Xcc; the RpfS sensor kinase binds DSF through an N-terminal PAS domain to control factors involved in the epiphytic phase of the disease cycle. Although DSF signalling has now been described in several *Xanthomonas* species and the related genera *Xylella* and *Stenotrophomonas*, it is not restricted to xanthomonads. DSF family signals are produced by the human pathogens *Burkholderia cenocepacia* and *Pseudomonas aeruginosa* where they act to regulate virulence, biofilm formation and antibiotic tolerance. Two sensors for DSF family signals have been described in *B. cenocepacia*: a membrane-associated histidine kinase unrelated to RpfC and a soluble PAS-GGDEF-EAL domain protein that couples DSF sensing to cyclic di-GMP turnover. In addition to intraspecies signalling, DSF family signals have been implicated in interspecies signalling that modulates bacterial behavior to include antibiotic tolerance. An understanding of these diverse signalling mechanisms could suggest strategies for interference, with consequences for disease control.

**FEMS-0964**

**Cell-cell communication / Quorum sensing: basic aspects**

**Quorum sensign in rhizobia: getting attached to their roots**

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**Background:** Most rhizobia have N-acyl-homoserine (AHL)-based quorum sensing regulatory systems. These influence phenotypes such as plasmid transfer, exopolysaccharide synthesis and processing, biofilm formation, motility and root attachment.

**Objectives:** In *Rhizobium leguminosarum* bv. *viciae*, which forms nitrogen-fixing nodules on peas and vetch, quorum-sensing regulation plays an important role in competitiveness for nodule infection. Our aim was to identify the key genes for this and their regulation.

**Methods:** We identified a small protein (CinS) that is co-expressed in a population-density-dependent manner with the AHL synthase CinI. CinS binds to the repressor PraR, thereby relieving repression of several genes including LuxR-type quorum-sensing regulators. Mutation of *praR* enhanced biofilm formation *in vitro* and on legume roots; this was correlated with enhanced infect infection of pea nodules in competition with the wild-type. Analysis of gene expression by microarrays and promoter fusions revealed that PraR represses its own transcription and the expression of several genes including those encoding secreted proteins (including the adhesins RapA2, RapB and RapC, a cadherin and the glycanase PlyB). Single mutations in *rapA2*, *rapB*, *rapC*, *plyB*, the cadherin or *rosR* did not affect the enhanced root attachment or nodule competitiveness of the *praR* mutant. However combinations of mutations in *rapA*, *rapB* and *rapC* abolished the enhanced attachment and nodule competitiveness.

**Conclusions:** Relief of PraR-mediated repression via quorum-sensing regulation determines a lifestyle switch allowing the expression of genes that are important for biofilm formation on roots and consequently enhances infection of legume roots and formation of nitrogen-fixing nodules.

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**FEMS-3159**

**Jorgen Lehmann and Gerhard Domagk event on *Mycobacterium tuberculosis*: current concepts of immunity in tuberculosis and their relevance for vaccine development**

**Towards new TB vaccines - what are the challenges?**

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To control the global threat from tuberculosis, we urgently need new TB vaccines that induce effective protection, as well as new drug regimens and diagnostic tools. However there are significant challenges. A new TB vaccine might replace the current BCG vaccine at birth, or be given as a boosting vaccine following BCG (or a BCG replacement vaccine) to infants or young adolescents. A post exposure vaccine could be given to older adolescents or adults. In addition, a therapeutic vaccine could be given to those with clinical disease to accelerate bacterial clearance. These vaccines could include improved BCG vaccines, safe live mycobacteria, antigens delivered by viral vectors, and proteins delivered with adjuvant. Vaccines could be injected or delivered by the mucosal route. There is a vaccine development pipeline with ongoing international efforts to coordinate and facilitate the development and testing of the most promising candidates. These efforts would be accelerated if we had biomarkers or protective biosignatures that predicted the protective efficacy of a new vaccine. Although there is increasing investment in non-human primate models, at present we also do not have animal models that reliably predict protection in man, so the main challenge is how to prioritise development of the best candidate vaccines. Although some measures of immunity have proved disappointing, there are promising developments exploiting gene expression signatures and novel mycobacterial growth inhibition assays. Identifying protective biosignatures, which may differ for different types of vaccines, would greatly facilitate the prioritisation of new TB vaccine candidates for further development. It would also be useful if we could understand better why the existing BCG vaccine induces such variable protection, to ensure that any new TB vaccine will work irrespective of location.

**FEMS-3156**

**Jorgen Lehmann and Gerhard Domagk event on *Mycobacterium tuberculosis*: current concepts of immunity in tuberculosis and their relevance for vaccine development**

**Steps ahead in the Development of New Vaccines Against Tuberculosis, searching for new attenuated vaccines**

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Steps ahead in the development of new vaccines against Tuberculosis, searching for the new attenuated vaccines

Carlos Martin

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One of the major challenges in the field of TB vaccines is the lack of a correlation of protection what necessary for efficacy evaluation of new vaccines in clinical trials in endemic countries. Live attenuated vaccine candidate MTBVAC is an attenuated *Mycobacterium tuberculosis* (MTB) genetically engineered to fulfil the Geneva consensus requirements to enter human clinical trials. We selected a MTB clinical isolate belonging to Euro-American lineage to generate two independent deletions without antibiotic-resistance markers. One is the *phoP*, essential for virulence in MTB where it controls expression of approximately 2% of the genes, including those for the ESX-1 secretion apparatus, a major virulence determinant and the secretion of the immunodominant Ag85 complex. Deletion in *phoP* gene lead to compromised production of pathogen-specific cell wall components and attenuation both *ex vivo* and *in vivo*. Deletion in *fadD26*, inactivate phthiocerol dimycocerosates (DIM) operon which, codes for the production of one of the major MTB virulence factors.

MTBVAC exhibits safety and biodistribution profiles similar to BCG and confers superior protection in preclinical studies. A major difference between MTBVAC and BCG, which is derived from the cattle pathogen *M. bovis*, is that BCG has many deletions in its genome when compared to MTB, and during the attenuation process BCG had lost over a hundred additional genes from its genome including immunodominant antigens. After more than 15 years of preclinical development MTBVAC is the first live attenuated vaccine to enter clinical evaluation in Lausanne

(Switzerland) with Prof Spertini as Principal Investigator and sponsored by Biofabri and TBVI (ClinicalTrials.gov: NCT02013245).

**FEMS-2577**

**Jorgen Lehmann and Gerhard Domagk event on *Mycobacterium tuberculosis*: current concepts of immunity in tuberculosis and their relevance for vaccine development**

**MYCOBACTERIUM MARINUM CAN CROSS THE BLOOD-BRAIN BARRIER VIA DIFFERENT MIGRATION ROUTES**

*L.M. van Leeuwen<sup>1</sup>, A.M. van Furth<sup>2</sup>, W. Bitter<sup>1</sup>, M. van der Kuip<sup>2</sup>, A.M. van der Sar<sup>1</sup>*

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**Background**

Tuberculous meningitis (TBM) is a major disease burden in children in TB endemic countries.

**Objectives**

We use our zebrafish – *Mycobacterium marinum* infection model for TBM to study early granuloma formation in brain and meninges of zebrafish embryos.

**Methods**

We dissected the migration mechanism employed by *M. marinum* to cross the blood-brain barrier (BBB) and cause infection of the central nervous system. To study the role of macrophages in this process, we depleted the macrophage pool in zebrafish embryos with pu.1 morpholinos and clodronate filled liposomes. Embryos lacking phagocytic cells showed an increased overall infection rate and a higher bacterial load in the head at 5 days post infection in comparison to the control group. Detailed analysis showed that in the presence of macrophages, *M. marinum* leaves the bloodstream and forms early granulomas consisting of mycobacteria and L-plastin positive phagocytes in brain tissue. Virtually all *M. marinum* inside and outside the blood vessels were present in phagocytic cells, suggesting that mycobacteria use macrophages as a Trojan horse to cross the BBB. As expected, we did not observe granuloma structures after macrophage depletion. However, we still found extravasated mycobacteria in the brain parenchyma. Most of these mycobacteria were found in close vicinity of blood vessels, following the blood vessel shape in a longitudinal manner.

**Conclusions**

We observed that *M. marinum* preferably use macrophages as Trojan Horses to cross the BBB. However, in the absence of macrophages *M. marinum* can adept and seems capable of using additional migration routes.

**FEMS-1678**

**Jorgen Lehmann and Gerhard Domagk event on *Mycobacterium tuberculosis*: current concepts of immunity in tuberculosis and their relevance for vaccine development**

## **THE ROLE OF A TYPE VII SECRETION CHAPERONE IN THE SPECIFIC SUBSTRATE RECOGNITION IN PATHOGENIC MYCOBACTERIA**

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### **Background**

Type VII secretion (T7S) is employed by mycobacteria, including the important pathogen *Mycobacterium tuberculosis*, to export protein effectors across their highly unusual cell envelope. *M. tuberculosis* contains five paralogous T7S systems, ESX-1 to ESX-5, each having its own role in viability and/or virulence. The mycobacteria-specific PE/PPE proteins are one group of proteins that are secreted via these secretion systems, of which at least some are secreted as heterodimers. We have previously shown that the cytosolic chaperone EspG specifically interacts with cognate PE/PPE dimers and is required for their successful secretion.

### **Objectives**

Our aim is to understand the determinant factors for substrate-specific binding of EspG to T7S substrates.

### **Methods**

The crystal structure of ESX-5 chaperone EspG<sub>5</sub> with the ESX-5 substrate pair PE25/PPE41 was determined and important residues for chaperone-binding of several PPE proteins were mutated. Additionally, EspG-binding domains of ESX-1 and ESX-5 substrates were exchanged.

### **Conclusions**

The structure revealed that EspG<sub>5</sub> interacts with the elongated end of PPE41, a region containing several hydrophobic residues that are conserved in PPE substrates of different secretion systems. Mutating these residues of multiple ESX-5 PPE substrates and an ESX-1 dependent PPE protein abolished the interaction with the cognate chaperone and blocked their secretion by *Mycobacterium marinum*. Subsequently, exchanging the complete EspG-binding domain between an ESX-1 and ESX-5 PPE protein altered chaperone-binding specificity. The effect of system-specific chaperone-binding on rerouting of T7S substrates is currently tested. In

summary, we have elucidated the molecular determinants for EspG-specific binding to PPE substrates and the key role of chaperone-binding in secretion.

**FEMS-0630**

**Jorgen Lehmann and Gerhard Domagk event on *Mycobacterium tuberculosis*: current concepts of immunity in tuberculosis and their relevance for vaccine development**

**MYCOBACTERIUM LEPRAE (M. LEPRAE) CREATES LIPID-RICH INTRACELLULAR ENVIRONMENT BY MODULATING EXPRESSION OF HOST GENES IN INFECTED MACROPHAGES**

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**Background**

*Mycobacterium leprae* (*M. leprae*), the causative agent of leprosy, parasitizes within lipid droplets stored in enlarged phagosome of histiocytes, which is thought to be important nutrient source and sheltering for the bacillus. However, the mechanisms by which *M. leprae* triggers lipid accumulation within phagosomes remain unclear.

**Objectives**

To elucidate the mechanism underlying *M. leprae*-induced lipid accumulation in macrophages.

**Methods**

Real-time PCR, western blotting, histochemical staining and DNA microarray were employed to analyze human premonocytic THP-1 cells infected with *M. leprae* as well as skin biopsies and smears of leprosy patients.

**Conclusions**

Live *M. leprae*, but not heat-killed *M. leprae*, specifically induced adipose differentiation-related protein (ADRP) and perilipin, which are essential regulators for lipid accumulation, in THP-1 cells. However, live *M. leprae* suppressed and inactivated hormone sensitive lipase (HSL). Clofazimine, one of the drugs for treating leprosy, devastated the *M. leprae*-induced lipid-rich intracellular environment by modulating the expression of ADRP and HSL, and by activating the innate immune response in host macrophages. Staining of skin biopsies and smears confirmed the above results. DNA microarray analysis revealed that live *M. leprae* modulated gene expressions important for lipid accumulation, as well as their common transcriptional factors, *i.e.* peroxisome proliferator-activated receptor (PPAR) $\delta$  and PPAR $\gamma$ . The antagonists of PPAR $\delta$  and PPAR $\gamma$  abolished the effect of *M. leprae* to modulate such gene expressions and lipid accumulation in macrophages. These results indicate that live *M. leprae* creates lipid-rich intracellular environment by modulating gene expressions of host macrophages through PPAR $\delta$  and PPAR $\gamma$  signaling.





## FEMS-1292

### Imaging of macromolecular structures

#### Structure and evolution of rotary motors

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Microbes propel themselves in beneficial directions using cell wall-embedded rotary motors that spin helical propellers. Bacteria swim using bacterial flagellar motors, large nanomachines composed of hundreds of proteins — from approximately twenty protein families — that harness proton-motive force to convey torque to a multi-micron flagellum, while archaea assemble "archaella" that utilize ATP hydrolyse to rotate an analogous filament. How do these function, and how did they evolve? To understand this we are using electron cryo-tomography to perform 3D in situ imaging of these motors. We have recently shown that bacterial flagellar motors are considerably more complex than the 'normal' core motor found in *Salmonella* and *E. coli*, thus shedding light on the evolutionary processes used to adapt macromolecular machinery. Why have some bacteria recruited additional proteins to a core that is already fully functional? I will describe phylogenetic and structural work to understand this question. We are currently focusing on a family of large novel structural components found in a variety of proteobacteria including the *Vibrio*, *Campylobacter*, and *Helicobacter* genera. By combining bacterial genetics, phylogenetics and electron cryo-tomography we have identified the proteins that form these large structures. Combining these results we have developed a model for the recruitment of these additional proteins, and have been able to speculate on the effect of these proteins on motor mechanics, in turn highlighting possible evolutionary driving forces behind protein recruitment. I will then describe recent work to study the analogous archaellar motor.

**FEMS-0591**

**Imaging of macromolecular structures**

**Cryo-EM of the Type VII secretion system in *Mycobacterium tuberculosis***

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We showed in Cell; 129:1287 that after prolonged infection in macrophages and dendritic cells, *M. tuberculosis* translocates from phago-lysosomes to the cytosol and killed the host cell a few days later, while the BCG vaccine strain failed to translocate. We found that this process was dependent on a gene in the extended RD1 region (ext-RD1). We then focused on BCG with a knock-in of the entire ext-RD1, which was recently identified by one of our former team member as a novel type VII secretion system (T7SS) (Abdallah AM et al., Nat Rev Microbiol. 2007). We found that these bacteria translocate to the cytosol of the host cell 7 days after infection. We concluded that the ESX-1 system is sufficient for translocation (Abdallah AM et al., J of Immunol. 2011 and Houben et al., Cell Microbiol. 2012).

Since my move to Maastricht University where I was invited to establish a new Institute for Nanoscopy ([www.maastrichtuniversity.nl/m4i](http://www.maastrichtuniversity.nl/m4i)) we are now using cryo-EM single-particle analysis (SPA), and cryo-EM tomography. We investigate recombinant purified proteins of individual gene products from the T7SS. In addition, we are purifying the entire intact T7SS structure using biochemical methods for 3D reconstruction. We will also generate lamellae of infected cells using cryo-FIB/SEM technology. This should lay the groundwork for the development of novel antibiotics and better vaccines. Since this FEMS meeting is next door to my new laboratory interested colleagues are welcome to contact me for a visit (Universiteitssingel 50, Room G0.201) while attending the meeting ([peter.peters@maastrichtuniversity.nl](mailto:peter.peters@maastrichtuniversity.nl)).

**FEMS-2842**

**Imaging of macromolecular structures**

**The assembly and mechanism of a bacterial drug efflux pump**

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Microorganisms encode several classes of transmembrane molecular pumps that can expel a wide range of chemically distinct toxic substances. These machines contribute to the capacity of the organisms to withstand harsh environments, and they help to confer resistance against clinical antimicrobial agents. In Gram-negative bacteria, the pumps comprise tripartite assemblies that actively transport drugs and other harmful compounds across the cell envelope. We describe recent structural and functional data that have provided insights into the architecture and transport mechanism of the AcrA-AcrB-TolC pump of *Escherichia coli*. This multi-drug efflux pump is powered by AcrB, a member of the resistance/nodulation/cell division (RND) family of transporters, which are energised by proton electrochemical gradients. Crystallographic data reveal how a small protein AcrZ is engaged in a concave surface in the transmembrane domain of AcrB, and we discuss how this interaction may affect the efflux activities of AcrB and other RND family members.

**FEMS-3165**

**Imaging of macromolecular structures**

**Structure and function of type 6 secretion and related assemblies**

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Interactions of a bacterial cell with other bacteria, eukaryotes or archaea are crucial for symbiosis, pathogenicity, competition and defense. These bacterial cell-cell interactions are often mediated by macromolecular machines. In evolution, a phage-derived bacterial contractile structure proved to be very successful in mediating cell-cell interactions in an either contact-dependent or indirect manner. We applied electron cryotomography in an interdisciplinary approach to understand the structure and mechanism of the type VI secretion (T6S) system, arrays of metamorphosis-associated contractile structures (MACs), and other bacterial contractile assemblies. State-of-the-art sample preparation techniques recently allowed us to image bacteria engaging with a eukaryotic host.

**FEMS-2839**

**Bacterial phosphorylation networks**

**New insights into the GacS multikinase network controlling virulence in *Pseudomonas aeruginosa***

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The GacS/A two-component signalling network of *Pseudomonas aeruginosa* plays a major role in controlling virulence. This network features multiple sensor kinases that work together to decide between acute and chronic modes of infection [1,2]. Signalling by the kinase GacS favours chronic infection, while another kinase, RetS, favours acute infection by inhibiting GacS. We have been investigating how RetS interferes with GacS function and we have found that there are three distinct ways in which RetS can inhibit GacS: 1) RetS can accept phosphoryl groups from GacS-P; 2) RetS has phosphatase activity towards GacS-P; and 3) RetS prevents autophosphorylation of GacS. We can selectively disable each of these mechanisms by introducing specific point mutations into RetS and have tested the effects of these mutations in a mouse model of respiratory infection. We find that loss of either mechanism 1 or 2 dramatically decreases acute virulence, while loss of both mechanisms 1 and 2 abolishes acute virulence. RetS function *in vivo* therefore depends upon its ability to bring about the dephosphorylation of GacS-P. This study reveals an unexpected degree of complexity in the intricacy of the interactions between the sensor kinases governing the choice between different modes of virulence in this important pathogen.

[1] Goodman et al. (2009) Genes Dev 23, 249;

[2] Ventre et al. (2006) Proc Natl Acad Sci USA 103, 171;

## FEMS-2849

### Bacterial phosphorylation networks

#### How do you Coordinate signals from two chemosensory pathways to control one flagellar motor?

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The chemotaxis pathway of *E.coli* is probably the best understood sensory pathway in bacteriology. A kinase, CheA, autophosphorylates in response to a decrease in attractant, and transfers a phosphoryl to either a diffusing single domain response regulator, CheY, which when phosphorylated binds the switch protein of the flagellar motor to cause a change in rotational direction, or phosphorylates a methyl esterase controlling receptor adaptation. However, many bacterial species have more than one chemosensory pathway controlling the direction of flagellar rotation.

The photoheterotrophic bacterium *Rhodobacter sphaeroides* expresses two pathways, one localised with transmembrane receptors to large patches on the membrane and one localised with soluble chemoreceptors, and associated with the chromosomes, segregating with the chromosomes on division to ensure each daughter inherits a cluster.

*In vitro* and *in vivo* methods, including phosphotransfer, fluorescence microscopy, cryo EM, molecular genetics, mass spectrometry, live cell tracking and mathematic modelling have been used to develop an understanding of how the direction of rotation of a single flagellar motor is controlled by integrating responses to extracellular and intracellular signals through the two pathways. Recent work suggests that signals from the cytoplasmic cluster dominate and *R. sphaeroides* tunes its swimming behaviour in response to current metabolic requirements.

## **FEMS-2813**

### **Bacterial phosphorylation networks**

#### **Signalling by two-component systems. A structural view**

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Two-component systems (TCS) are the predominant signal transduction mechanism in bacteria, are present in lower eukaryotes such as fungi and plants, but they are absent from mammals. The key players in these systems are two proteins, histidine kinases (HKs) along with response regulators (RRs) which transmit signals through phosphoryl-transfer. HKs are sensor dimeric proteins that, upon signal detection, bind ATP and autophosphorylate in a catalytic histidine residue. Transfer of the phosphoryl group from the HK histidine to a conserved aspartic of its cognate RR propagates the signal and triggers the cellular response. Signal cease by the auto or HK-mediated de-phosphorylation of the RR. The catalytic machinery in charge of carrying out the reactions related with signaling process, either in the HK or the RR, is highly conserved and the couple interaction shows high-fidelity. Despite being the more abundant signal transduction systems in the nature, the molecular bases of these reactions are neither fully understood at biochemical level nor well-characterized at structural level, as HK autophosphorylation reaction is known to be conducted via a *cis* or *trans* mechanism in different HKs or the HK-RR phosphoryl transfer and HK-mediated RR de-phosphorylation have been demonstrated not to be reversible. In an effort to clarify the molecular basis of TCS signaling we have worked during the last 10 years with HK853-RR468 and EnvZ-OmpR, two prototypical TCS. Our functional and structural results, which will be discussed in the presentation, shed light on the molecular basis of each reaction and their interrelationship with HK-RR recognition and specificity.

**FEMS-2856**

**Fungal cell biology**

**Molecular motors in spatially organizing the fungal cell**

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To cause plant disease, pathogenic fungi can secrete effector proteins into plant cells to suppress plant immunity and facilitate fungal infection. Most fungal pathogens infect plants using very long strand-like cells, called hyphae, which secrete effectors from their tips into host tissue. How fungi undergo long-distance cell signalling to regulate effector production during infection is not known. Here, I show that long-distance retrograde motility of early endosomes (EEs) is necessary to trigger transcription of effector-encoding genes during plant infection by the pathogenic fungus *Ustilago maydis*. This motor-dependent retrograde EE motility controls effector production and secretion during host cell invasion. It involves the mitogen-activated kinase Crk1, which travels on EEs and participates in control of effector production. Fungal pathogens therefore undergo endosome-mediated long range-signalling to orchestrate host invasion. These findings add to recent discovery that EEs distribute the machinery for protein translation. Thus, 15 years after the discovery of motile endosomes in fungi, the biological role of their motility is beginning to reveal.



**FEMS-2791**

**Fungal cell biology**

**Peroxisome biogenesis and proliferation in yeast**

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Peroxisomes are cell organelles that are present in almost all eukaryotic cells. They can harbor a large variety of enzymes, which highly depends on the organism, tissue and developmental stage. Common peroxisomal functions include the beta-oxidation of fatty acids and hydrogen peroxide metabolism. The importance of these organelles is illustrated by the fact that peroxisome malfunction results in severe disorders and is often lethal in man.

In yeast and filamentous fungi peroxisomes play a role in the metabolism of various unusual carbon and organic nitrogen sources, such as alkanes, fatty acids, methanol, primary amines and uric acid. Notably, in filamentous fungi peroxisomes are crucial for the biosynthesis of commercially important metabolites, such as beta-lactam antibiotics.

We study peroxisome biology using *Saccharomyces cerevisiae* and *Hansenula polymorpha* as model organisms. An important and highly debated topic is how new peroxisomes are formed. One model proposes that peroxisomes are semi-autonomous organelles that originate by fission of pre-existing ones, similar to mitochondria. According to an alternative model peroxisomes form de novo from the endoplasmic reticulum, which implies that peroxisomes form a branch of the endomembrane system.

We recently re-investigated both pathways using advanced electron and fluorescence microscopy techniques. Our data suggest that in yeast peroxisome fission is the major mode of peroxisome proliferation.

**FEMS-2897**  
**Fungal cell biology**

**Genetic Networks in Budding Yeast**

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To define the general principles of genetic networks, our group developed a functional genomics platform called 'synthetic genetic array' (SGA) analysis that automates yeast genetics and enables the systematic construction of double and triple mutants. One of our major goals has been to use a simple phenotypic readout of cell growth rate – colony size – to produce the first complete genetic interaction map for any cell, and to empirically delineate the properties of genetic networks. We have now analysed the majority of all possible 18 million yeast gene pairs. The resultant network consists of ~560,000 genetic interactions, spanning 93% of all yeast genes. Analysis of the network has revealed: [1] a central role for and unique properties of essential genes; [2] hubs and pleiotropic genes on the network which show a clear association with several fundamental physiological and evolutionary properties that are predictive of genetic interactions in other organisms; [3] functional modules that we use to predict and test conservation of interactions in other systems.

**FEMS-2850**

**Fungal cell biology**

**The importance of fungi and their movement in crops**

**FEMS-1033**

**Education**

## **MICROBIO: ON LINE TOOLS FOR TEACHING AND DISSEMINATION MICROBIOLOGY CONCEPTS**

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### **Background**

Teaching and research are main jobs of a university professor. Both tasks should be nourished by public dissemination of scientific matters. Under the acronym of “**microBIO**” I have group together several on line tools for the dissemination and spread news and curiosities about microbiology and science in general.

### **Objectives**

In an understandable and entertaining way, with scientific accuracy, the goals of **microBIO** are: i) make science and microbiology understandable to all kind of people, ii) wake up the curiosity for microbiology and arouse young scientific vocations, iii) avoid the isolation of scientist and increase the visibility of our work.

### **Methods**

**microBIO** get together several tools on line, open and free: blog, twitter, Facebook, Scoop.it, Storify, YouTube channel, TEDx talks,... We use social networks to disseminate and share images, teaching resources, videos, links and all kind of scientific information of interest.

### **Conclusions**

Two experiences have special attention. 1) Massive Online Open Courses (MOOC) as introductory courses of general microbiology and virology: “Microbes around us” and “Pandemics and new viral infections”, both for people with interest in scientist topics, primarily undergraduate students, first-year university course, science teachers and journalists; ii) microbiology master classes by twitter, during 8 weeks, once a week, we share around 30 “tweets” with the hashtag #microMOOC. In only 140 characters we taught different subjects related with microbiology.

**microBIO** has thousands of followers from all over the world, 37% from Spain and

the remainder from other countries: México, USA and Latin-America. **microBIO** demonstrates that microbiology can also be “trending topic”.

**FEMS-1060**

**Education**

## **DEVELOPING EUROPEAN COMMON CURRICULUM FOR MICROBIOLOGY EDUCATION: IS THERE A NEED?**

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### **Background**

Microbiology including General and Applied Microbiology is main subject in Biology (Microbiology) education at European universities. Microbial Biotechnology is important for training in Biology education and Clinical Microbiology - Medicine one. American Society for Microbiology is developing a curriculum. European common curriculum would be of significance to train specialists on the unified high level.

### **Objectives**

The motivations to train microbiologists in European countries would be clarified; the role of Microbiology in Biology education and main parts in Microbiology content should be defined.

### **Methods**

Education practice in Bologna process framework as well as American, European national or university curriculums would be compared and analysed.

### **Conclusions**

To have European common curriculum the following would be of significance:

- for motivations: description and preservation of wide biodiversity, including microorganisms, in different regions in Europe; isolation, identification and study of microorganisms in different ecological environment, discovering their role in nature; monitoring and control of potentially dangerous microorganisms in nature and among habitants, developing new strategy against endemic pathogens; using microorganisms in food and pharmaceutical industry; developing bio-energy production; control of biosafety; biology education at secondary schools.
- Three modules are significant for education: Biodiversity, including biology of microorganisms; Physiology, including microbial physiology; Physico-Chemical

Biology. General (Basic and Systematic) Microbiology and Microbial Biotechnology and/or Clinical Microbiology can be recommended as main topics.

- For teaching issues improving computer and lab works would be efficient in learning Microbiology and developing appropriate practical skills.

To have acceptable curriculum and to implement in different countries, adoption by FEMS (Education Group) would be important.

**FEMS-2642**

**Education**

## **DELIVER MICROBIOLOGICAL CONCEPTS TO A GENERAL AUDIENCE WITH AN ANIMATED CARTOON**

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<sup>3</sup>*Center for Biofilm Engineering, Montana State University, Bozeman, USA*

### **Background**

As stated by the European Charter for Researchers, scientists should ensure that their research activities are made known to society at large in such a way that they can be understood by non-specialists, thereby improving the public's understanding of science (EC 2005). Science communication is considered a fundamental tool to promote science study, to raise the visibility of universities and their research groups, and to influence policy decisions and funding opportunities.

### **Objectives**

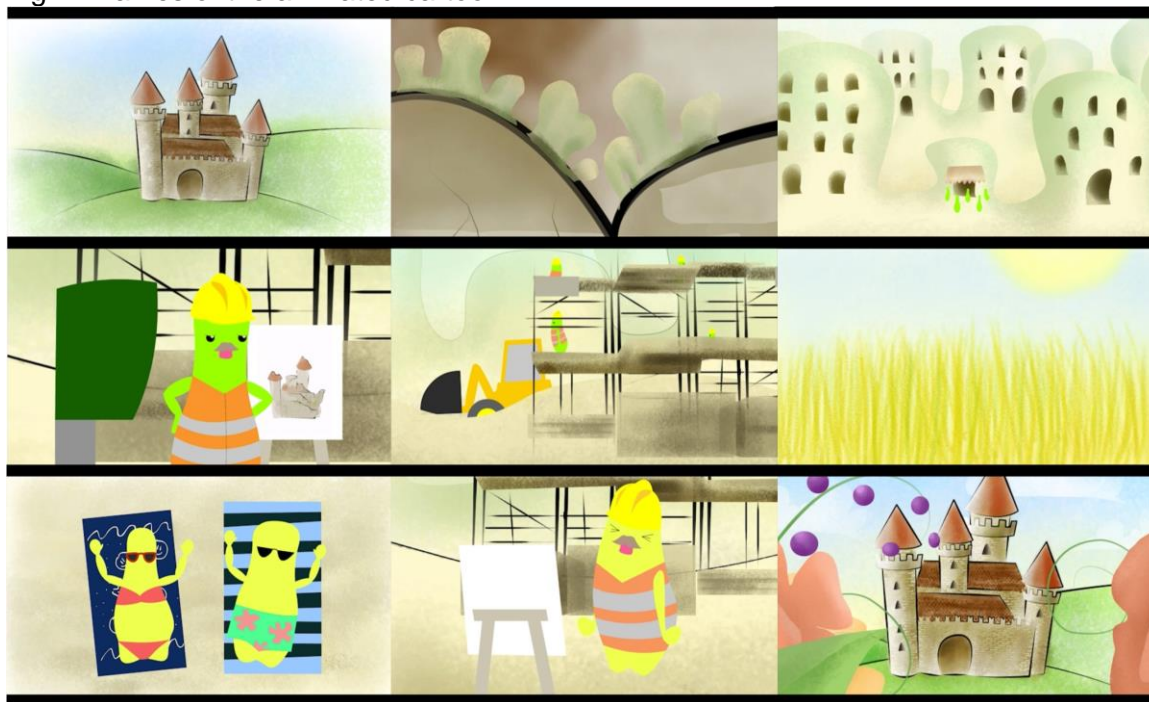
The aim of this work was to create an animated cartoon to raise awareness among young people, citizens, end-users and policy makers about the significance and the broad impacts of the EU funded project ESENCYA "Environmental SENSory perception in CYAnobacterial biofilms: understanding biodeterioration of outdoor stone materials in a changing environment".

### **Methods**

By working with a multifunctional team composed of character designers, visual development artists, communication specialists and scientists, we created a visually engaging story about complex concepts in new and innovative ways (Fig. 1). We translated complex information and ideas into intuitive and effective visual explanations, and we used a narrative strategy to get our message across, clearly and concisely while reducing misunderstanding.



Fig. 1: frames of the animated cartoon.



## Conclusions

This animated cartoon wants to encourage and inspire actions directed towards raising public awareness of the importance of sciences for our contemporary society in a cultural and historical perspective. The microbiology community, in Europe and around the world, can find ideas, blueprints and suggestions for activities directed towards raising public awareness of science, technology and other fields where microbiology plays a strong role.

**FEMS-1167**

**Food biotechnology**

***Clostridium botulinum* and the safety of modern foods**

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Foodborne botulism is a severe and often fatal neuromuscular intoxication caused by consumption of neurotoxin formed by strains of *Clostridium botulinum* (and very occasionally by strains of *C. baratii* or *C. butyricum*). *C. botulinum* is defined on the basis of one physiological property, an ability to form botulinum neurotoxin. It is a heterogeneous species that is separated into four distinct phylogenetic/physiological groups (*C. botulinum* Groups I to IV). The distinction between each Group is sufficient to merit separation into different species. Seven botulinum neurotoxins (types A to G), and >30 neurotoxin sub-types are recognised. The botulinum neurotoxins are the most potent toxins known; 50ng of neurotoxin may cause human illness and possibly death.

*C. botulinum* Groups I and II and the neurotoxins they form (types A, B, E and F) are responsible for most cases of foodborne botulism. These two physiologically distinct bacteria present different problems. *C. botulinum* Group I (proteolytic *C. botulinum*) is a mesophile (minimum growth temperature 10°C-12°C) that forms highly heat resistant spores, while *C. botulinum* Group II (non-proteolytic *C. botulinum*) is a psychrotroph (minimum growth temperature 2.5°C-3.0°C) that forms moderately heat resistant spores.

Recent advances in genomics have increased understanding of the biology of *C. botulinum* Groups I and II, while developments in mathematics have improved risk assessments. The safe development of novel foods and new food processes requires strong control measures for *C. botulinum* Groups I and II. The extreme severity of foodborne botulism ensures that regulators and industry remain vigilant to minimize the foodborne botulism risk.

**FEMS-2426**

**Food biotechnology**

**NEW INSIGHT INTO BIOGENIC AMINES: TOWARDS SOLUTION AND BIOTECHNOLOGICAL APPLICATIONS**

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**Background**

Biogenic amines (BA) are low molecular weight nitrogenous compounds mainly formed by the microbial decarboxylation of amino acids. The primary relevance of BA is that the consumption of foods or beverages containing high concentration of BA may cause food intoxication. Dairy products are among the foods likely to contain the highest concentrations of BA, being lactic acid bacteria the main BA-producers.

**Objectives**

Increase our knowledge on BA synthesis in order to adopt strategies for surveillance and prevention of BA accumulation in foods and towards the development of new biotechnological tools.

**Methods**

Strategies to reduce the BA content in fermented products:

- Avoid the presence of bacteria synthesizing BA through the food chain. The identification of BA gene clusters has allowed the design of molecular methods to identify and quantify the presence of BA producers in cheese samples.
- Identify key factors which favor BA accumulation. Different physicochemical and technological factors have been studied in order to determine their impact on BA accumulation either affecting bacterial growth or BA gene expression.
- Recently, the use of biogenic amine-degrading microorganisms has been proposed. It is necessary to identify and select highly competitive adjunct cultures capable of in situ reduce the content of BA during the elaboration of fermented products.

Beside of this, understand the transcriptional regulation of BA synthesis clusters offer opportunities for the development of new expression vectors.

**Conclusions**

The study of BA synthesis, is needed to propose new strategies to avoid BA accumulation in foods and can be used for the development of new biotechnological tools.

**FEMS-2374**

**Food biotechnology**

**THE PHAGE ENDOLYSIN LYSTP712 REQUIRES A FUNCTIONAL HOST MEMBRANE PROTEASE FTSH TO LYSE LACTOCOCCAL HOST CELLS**

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<sup>2</sup>Gut Health and Food Safety Programme, Institute of Food Research, Norwich, United Kingdom

**Background**

Bacteriophages are often the cause of milk fermentation failures and selecting phage resistant starters, namely *Lactococcus lactis* which is widely use in cheese manufacturing, may reduce the risk. *L. lactis* lacking the membrane protease FtsH became resistant to the phage TP712. In these mutants, viable phages amassed in the cytoplasm but could not be released.

**Objectives**

Lysis of the phage host is usually accomplished by the action of two phage proteins: a holin that disrupts the cytoplasmic membrane and an endolysin that degrades the peptidoglycan. Our objective was to demonstrate if the activity of any of these proteins was compromised by the absence of FtsH.

**Methods**

TP712 lysogens were induced with mitomycin C and treated with ionophores to simulate holin activity. Contrary to the wildtype strain, lysis was not triggered in *L. lactis*  $\Delta$ ftsH, pointing to a failure of the endolysin to degrade the cell wall. However, zymograms revealed the presence of an active LysTP712 endolysin in *L. lactis*  $\Delta$ ftsH lysogens. A fusion protein consisting of the fluorescent mCherry protein and the cell wall binding domain of LysTP712 fused to its C-terminus was synthesized in *E. coli* and purified. Binding experiments to whole cells demonstrated that the fusion protein localized at the septum in both WT and  $\Delta$ ftsH lactococcal cells but the latter bound 40% less protein.

**Conclusions**

Absence of FtsH does not compromise proper functioning of the TP712 holin.

LysTP712 synthesized in *L. lactis*  $\Delta$ ftsH is active *in vitro* but its binding to *L. lactis*  $\Delta$ ftsH cells is reduced.



**FEMS-2144**

**Food biotechnology**

## **TRANSGLUTAMINASE APPLICATION IN WHEAT BREAD PRODUCTION**

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<sup>3</sup>Department of Biotechnology Microbiology and Food Evaluation,

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### **Background**

Transglutaminase is used in different branches of the industry because of its ability to modify physicochemical properties of proteins. It can be the solution to many technological problems related to appropriate efficiency and food texture. Transglutaminase catalyses the formation of cross-links both within a protein molecule and between molecules of different proteins. In bakery industry it was found that at certain level of transglutaminase concentration it has a significant influence on the dough by increase of relaxation time of doughs. Taking into consideration fact that high relaxation times in general correspond to a high quality flour it is believed that transglutaminase would help improve the quality of weak flours.

### **Objectives**

The aim of the experiment is to determine transglutaminase influence on wheat bread quality.

### **Methods**

Transglutaminase was obtained from *Streptoverticillium mobaraense* strain KKP 2013 obtained from Culture Collection of Industrial Micro-organisms. Preparations were sprayed dried on maltodextrin as carrier. In the experiments there were used wheat flour type 500, two preparations of transglutaminase in four level of addition: 0.01%; 0.05%; 0.25% and 0.5. All physicochemical parameters were determined according to Polish and European Standards.

### **Conclusions**

Taking into consideration crumb characteristics there has been shown an increase in hardness and chewiness, explained as a result of an improvement in the structure of the crumb in comparison to a control breads. The quality of bread in the organoleptic

evaluation was not very diverse. Although the bread with the addition of transglutaminase received higher number of points than the bread without enzyme.

**FEMS-0574**

**Food biotechnology**

## **OXIDATION OF FERULIC ACID TO FORM DIMERS WITH HIGHER ANTIOXIDANT CAPACITY**

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<sup>1</sup>*fse, lautech, ogbomosho, Nigeria*

### **Background**

Ferulic acid is an abundant and widespread cinnamic acid derivative with antioxidant properties. However, its antioxidant activity is generally low when compared to conventional antioxidants, such as butylated hydroxyanisole (BHA) and other hydroxyl-cinnamic acids, such as sinapic acid. Laccases (EC 1.10.3.2) are enzymes that are capable of catalysing the oxidation of various phenolic compounds with the production of water as the only by-product, and are therefore often referred to as 'green' enzymes. The use of laccases in the oxidative coupling and polymerisation of phenolic compounds offers potential for the amplification of the antioxidative capacity of these phenolic compounds.

### **Objectives**

This work employed the use of laccase produced from *Trametes pubescens* to catalyse the modification of ferulic acid as a way of enhancing antioxidant capacity.

### **Methods**

An airlift reactor was used to produce laccase from free cells of *Trametes pubescens* and the laccase was purified. Reaction of ferulic acid (10mM) with laccase (10 U) was done at 28°C in a biphasic system of ethyl acetate and 100mM sodium acetate buffer pH 5.0. Products were separated, purified, characterized and antioxidant activities were determined.

### **Conclusions**

This work elucidates the structure of two dimers formed from ferulic acid in organic media one of which is more potent than ferulic acid in terms of antioxidant activity. This new product could find useful application in health and cosmetic industry.



**FEMS-2884**

**Signalling, small RNAs and regulation: virulence aspects**

**RNA-mediated regulatory circuits link stress response, adaptation and virulence in *Staphylococcus aureus***

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<sup>2</sup>INSERM E0230, University of Lyon, Lyon, France

*Staphylococcus aureus* is a remarkable versatile pathogen, able to cause a wide spectrum of human diseases, and is one of the main causes of community as well as hospital-acquired infections. The contribution of regulatory RNAs in the establishment of virulence in this pathogen is increasingly appreciated. Our previous data emphasize the multitude of regulatory steps affected by RNAIII in establishing a network of *S. aureus* virulence factors. We show that RNAIII coordinately represses the expression of numerous mRNAs that encode the transcriptional repressor of toxins, several virulence factors acting early in the infection process, and enzymes involved in peptidoglycan metabolism. Besides RNAIII, we demonstrated that the *S. aureus* genome likely encodes a high diversity of RNAs including *cis*-acting regulatory regions of mRNAs, *cis*-acting antisense RNAs, and small RNAs. One of these sRNAs attenuates the severity of acute systemic infections and enhances chronic catheter infection by regulating MgrA, a transcriptional regulator of capsule formation. Because *S. aureus* is essentially a human commensal, we propose that RsaA has been positively selected through evolution to support commensalism and saprophytic interactions with the host. We will also illustrate how regulatory proteins and RNAs form complex regulatory circuits to express the virulence factors in a dynamic manner.

References : Romilly et al. PLoS Pathog. 2014 10(3):e100397

**FEMS-2860**

**Signalling, small RNAs and regulation: virulence aspects**

**RNA-mediated regulation of Legionella virulence**

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*Legionella pneumophila*, a gram-negative intracellular pathogen, has evolved complex mechanisms to efficiently enter, replicate inside, and evade their hosts, aquatic amoebae or alveolar macrophages during disease. *L. pneumophila* has been shown to cycle between an infectious, non-replicating form thought to promote transmission to a new host, and an intracellular, replicative form, which does not express transmission/virulence traits. This biphasic lifecycle is tightly regulated by a complex regulatory cascade, implicating two component systems, sigma factors, RNA binding proteins and small ncRNAs. Here we have employed RNAseq, transcriptional start site (TSS) mapping and RIPseq combined with molecular biology approaches to better understand this regulatory network and the implication of ncRNAs. We first established a genome-wide map of the TSSs and operons for *L. pneumophila*, and identified over 500 new small ncRNAs that may act as potential regulators of cis- and trans-encoded target messenger RNAs. Among those a high amount of antisense transcription was observed. A detailed search for targets of the RNA-binding protein CsrA, a key regulator in the expression of the virulence phenotype of *L. pneumophila* led to the identification of over 50 mRNAs regulated by CsrA, many of which are virulence related proteins. Taken together, we identified the mRNA targets of the RNA binding protein CsrA, ncRNAs regulating CsrA as well as ncRNAs implicated in virulence. Furthermore we showed that tandem promoters and sRNAs with alternative usage are a hallmark of the life cycle depend virulence gene expression of *L. pneumophila*.

**FEMS-0649**

**Signalling, small RNAs and regulation: virulence aspects**

## **REGULATION OF THE TYPE I-F CRISPR-CAS SYSTEM OF PECTOBACTERIUM ATROSEPTICUM**

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### **Background**

The CRISPR-Cas 'prokaryotic adaptive immune system' represents an intricate defence strategy allowing bacteria to protect themselves against invading genetic elements such as bacteriophages or plasmids. Through the acquisition of short segments of invading nucleic acid sequences into genomic CRISPR loci, this system provides a heritable genetic memory of previous exposure and, in combination with various CRISPR-associated (cas) proteins, a means by which the bacteria can specifically recognise, target and eliminate the exogenous threat. In the past 10 years since the discovery of CRISPR-Cas systems, there has been a heavy focus on functionality; how the acquisition of genetic material occurs and how the system coordinates its targeted response. In contrast, very little is known how about how CRISPR-Cas systems are regulated and of the limited literature available, it seems that a universal mode of regulation is perhaps unlikely.

### **Objectives**

In this study, we focused on elucidating the regulatory control of the model Type I-F system in *Pectobacterium atrosepticum*.

### **Methods**

Unbiased transposon mutagenesis of a cas1 reporter strain was utilised to identify mutants exhibiting differential promoter activity compared with a wildtype control. CRISPR-Cas activity was also assessed in these mutant strains via conjugation efficiency and spacer acquisition assays.

### **Conclusions**

We have identified a network of regulators of CRISPR-Cas expression, highlighting a link to global regulators that sense the nutritional status of the cell. In addition, we have shown that mutating these regulators drastically affects both the primed acquisition and interference stages within CRISPR-Cas-mediated defence.



**FEMS-0794**

**Signalling, small RNAs and regulation: virulence aspects**

## **PROTEOLYSIS OF ANTI-SIGMA FACTORS IN THE TRANSDUCTION OF THE SIGNAL AND ACTIVATION OF EXTRACYTOPLASMIC FUNCTION SIGMA FACTORS**

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### **Background**

Regulation of gene expression allows bacteria to adapt to alterations in their environment. This regulation occurs mainly at the level of transcription initiation by modifying promoter recognition of the RNA polymerase, a recognition that is primarily modulated by substitution of the RNAP sigma subunit. Besides a primary sigma factor responsible of the expression of general functions, most bacteria contain alternative sigma factors that target expression of functions required only under specific circumstances. Of those, the extracytoplasmic function sigma factors ( $\sigma^{\text{ECF}}$ ) represent the largest group, and control expression of bacterial functions as important as stress responses, iron uptake and virulence.

### **Objectives**

Activation of  $\sigma^{\text{ECF}}$  is a tightly regulated process that usually occurs in response to environmental signals, a control that is carried out by anti-sigma factors. Binding of the  $\sigma^{\text{ECF}}$  to the RNAP and the anti-sigma factor is mutually exclusive. This work focusses on understanding the molecular mechanism behind  $\sigma^{\text{ECF}}$  activation.

### **Methods**

We have used *Pseudomonas* as bacterial model and a number of in vivo and in vitro techniques to monitor gene expression and protein levels, stability and structure.

### **Conclusions**

Activation of  $\sigma^{\text{ECF}}$  in response to the inducing signal requires the processing of the anti-sigma factor by a complex proteolytic cascade that involves, amongst other, the Prc and RseP proteases. Interestingly, several anti-sigma factors undergo self-cleavage prior to signal recognition by an enzyme-independent process. This cleavage produces two functional domains that interact and function together to transduce the presence of the signal and to activate the  $\sigma^{\text{ECF}}$ . The biological significance of this widespread mechanism is discussed.



**FEMS-0804**

**Signalling, small RNAs and regulation: virulence aspects**

**RESPONSE OF YEDQ DIGUANYLATE CYCLASE TO PYRIMIDINE NUCLEOTIDES BY DIRECT INTERACTION WITH THE METABOLIC ENZYME ASPARTATE CARBAMOYLTRANSFERASE AND ITS METABOLIC PRODUCT N-CARBAMOYL-L-ASPARTATE**

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**Background**

In *Escherichia coli*, formation of multicellular communities is mediated by the production of adhesion factors such as curli fibres and the extracellular polysaccharide cellulose. Synthesis of curli fibres and cellulose is co-regulated through the activity of the transcriptional factor CsgD and is under the control of several extracellular and intracellular signals, including the second messenger c-di-GMP. We demonstrated that curli and cellulose production are tightly linked to pyrimidine biosynthetic pathways. Indeed, transcription of curli operons is strongly affected by pyrimidine nucleotide availability; in addition, we found that exogenous uracil can trigger cellulose production independently of CsgD, by increasing c-di-GMP levels via the YedQ diguanylate cyclase.

**Objectives**

Through a combined *in vivo* and *in vitro* approach we studied the molecular mechanism underlying the uracil-dependent regulation of YedQ.

**Methods**

YedQ protein interactors were co-purified using his-tag pull-down technique and then identified through MudPIT technology. Interactions were validated using bacterial two-hybrid assay. Protein ligand binding was determined through differential scanning fluorimetry, while gene expression was measured with qRT-PCR assay.

**Conclusions**

We found that YedQ can directly interact with aspartate carbamoyl-transferase (PyrBI), an essential enzyme involved in pyrimidine nucleotides biosynthesis, in a manner dependent on uracil availability, and can also bind N-carbamoyl-L-aspartate the product of PyrBI enzyme. We hypothesise that aspartate carbamoyltransferase

and/or N-carbamoyl-L-aspartate can inhibit YedQ diguanylate cyclase activity by directly interacting with its cytoplasmic domain and this negative effect can be reversed by uracil. The YedQ/PyrBI interaction might represent a model of how activity of metabolic pathways can be coupled to a c-di-GMP signalling system.



**FEMS-1441**

**Signalling, small RNAs and regulation: virulence aspects**

**A NEW TARGET FOR THE SRNA OMRA: THE VIRF MRNA IN SHIGELLA FLEXNERI**

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**Background**

VirF is a transcriptional regulator which plays a central role in controlling virulence gene expression in *Shigella* spp, the ethiological agent of bacillary dysentery in humans. VirF protein is present in two different forms, independently translated. VirF<sub>30</sub> is responsible for activation of the virulence system and some chromosomal genes, whereas VirF<sub>21</sub> exerts negative feedback control on *virF* expression itself.

**Objectives**

This work investigates the role of OmrA, a sRNA induced under high osmolarity conditions, on the translation of VirF<sub>21</sub> and its impact on *Shigella* virulence.

**Methods**

In vitro translation, translational lacZ –fusions, OmrA overexpression were used to evaluate the effect of OmrA on VirF<sub>21</sub> translation. RNA footprinting was performed to map the OmrA binding site on *virF* mRNA.

**Conclusions**

We have provided evidence supporting the involvement of OmrA in the translational regulation of *Shigella* VirF. In particular, we have shown that under high osmolarity conditions the expression of a VirF<sub>21</sub> fusion is decreased severely in *E.coli* wt as compared to a defective *omrBA* derivative. Overexpression of OmrA, but not of OmrB, leads to a strong decrease in VirF<sub>21</sub> translation. We also performed RNA footprinting showing the precise binding site of OmrA on *virF* mRNA. These data suggest that OmrA might interfere with the translation of the *virF* mRNA, reducing the amount of VirF<sub>21</sub> peptide. The result of this complex regulation is a fine-tuned expression of virulence genes, allowing *Shigella* a prompt adaptation to different environments and conditions.



## **FEMS-1000**

### **Fungal human pathogens**

#### **Components of the fungal cell wall that turn on and turn off the immune inflammatory response**

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The fungal cell wall defines the perimeter of its own self and a boundary of communication with other organisms with which it interacts. For a fungal pathogen, every component and layer of the cell wall plays a specific role in its interactions with cells of the human host. We have tried to peel the functions of cell wall components by combining genetics, biochemistry and immunology to understand how the wall is assembled and how its constituents are recognized by the human immune system. This work has shown how the polysaccharides of the cell wall activate both the inflammatory and anti-inflammatory responses of the innate immune system.

Chitin is an essential polysaccharide of the walls of all fungal pathogens and the exoskeleton and eggs of invertebrate parasites. We identified NOD2, TLR9 and the mannose receptor as three essential fungal chitin-recognition receptors of innate immune cells and showed that the activation of NOD2/TLR9 receptors by low concentrations of small chitin particles, generated via the action of human chitinases, leads to selective secretion of the anti-inflammatory cytokine IL-10. In contrast, higher concentrations of chitin particles promoted a pro-inflammatory response via dectin-1-TLR2 signalling. NOD2 and TLR9 polymorphisms are associated with susceptibility to inflammatory conditions such as Crohn's disease, allergy and asthma. Chitin therefore promotes size-dependent anti- and pro-inflammatory immune responses that are critical for immune homeostasis, but can also promote the development of asthma and allergy.

#### **References:**

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**FEMS-1242**

**Fungal human pathogens**

**Pathogenicity and immune evasion of the human-pathogenic fungus**

***Aspergillus fumigatus***

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(HKI) and Institute of Microbiology, Friedrich Schiller University Jena, Germany

## **Background**

The filamentous fungus *Aspergillus fumigatus* is the most important air-borne fungal pathogen causing 90 % of all systemic *Aspergillus* infections in humans. The most severe disease caused by *A. fumigatus* is invasive aspergillosis which almost exclusively occurs in immunocompromised patients. A lack of reliable diagnostic tools and effective treatment options results in high mortality rates despite therapy.

## **Objectives**

We aim at identifying pathogenicity determinants and mechanisms how *A. fumigatus* can overcome the response of immune effector cells.

## **Methods**

Transcriptome, (immune)proteome analyses including systems biological analyses with subsequent generation of deletion mutants and their analysis in processing by the immune system like neutrophilic granulocytes, alveolar macrophages and complement; test of mutants in a mouse infection model.

## **Conclusions**

*A. fumigatus* has developed immune evasion mechanisms which interfere at the different levels of the infection process with the response of the human host. These include recognition of conidia, modulation of phagocytosis, intracellular processing, neutrophil extracellular trap formation, and complement activation. We have identified several molecules including surface components such as proteins and dihydroxynaphthalene melanin as well as secondary metabolites, which manipulate the immune response.

## **References**

- (1) Scharf et al. (2014) *PLoS Pathogens* 10: e1003859.
- (2) Bacher et al. (2014) *J Immunology* 193: 3332-3343.

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**FEMS-0769**

**Fungal human pathogens**

**FIRST METABOLIC NETWORK OF THE SEBORRHEIC DERMATITIS  
ASSOCIATED YEAST *MALASSEZIA FURFUR***

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**Background**

Yeasts in the genus *Malassezia* are part of the skin microbiota of humans and other mammals. These commensal yeasts are related to several disorders including dandruff, seborrheic dermatitis and pityriasis versicolor. However, little is known about the pathogenesis process of *Malassezia* and its metabolism in these skin diseases, especially at the molecular level.

**Objectives**

In this study, we aim to describe the characteristics of *Malassezia furfur* genome-scale metabolic network

**Methods**

For this, a bioinformatics annotation methodology and a (LC/MS)-based shotgun proteomics profiling were used to characterize the 15.7 Mb genome and the subsequent assignment of the reactions and metabolites.

**Conclusions**

A total of 757 proteins were found in the proteomic profiling and were used with previous reports to annotate the genome, achieving a first draft of the metabolic network of this yeast. Metabolic reconstruction predicts an interesting degeneracy on the objective function, reflecting several mechanism for the yeast survival as we identified a relatively low number of genes related to lipid biosynthesis but several genes encoding lipid-hydrolyzing enzymes. These enzymes that could have a role in the pathogenesis of the yeast.

**FEMS-0722**

**Fungal human pathogens**

**IMMUNIZATION WITH ANTIGEN DISPLAYED MICROBIAL CELLS BY MOLECULAR DISPLAY TECHNOLOGY AGAINST CANDIDIASIS**

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**Background**

The administration of caspofungin, micafungin, anidulafungin, and amphotericin B are often involved in pharmacotherapy of candidiasis. Unfortunately, mutants of *Candida* species with reduced susceptibility to these drugs have emerged. Therefore, prevention of infection by vaccination against *Candida* species is thought to be important complementary method to pharmacotherapy of candidiasis.

**Objectives**

*Candida albicans* enolase 1 (Eno1) has been reported a lot as an immunodominant antigen. To develop a convenient and novel type of vaccine against candidiasis, we designed microorganism cells displaying Eno1p as a model antigen on *Saccharomyces cerevisiae* and *Lactobacillus casei* surfaces, respectively [1,2].

**Methods**

The Eno1p-displayed *S. cerevisiae* and *L. casei* cells were constructed by molecular display technology [3]. Eno1 protein was genetically fused to each cell wall proteins to be displayed on these surfaces. Delivery of the cells to mice by oral or intranasal administration increased the titers of anti-Eno1p antibodies and prolonged the lives of mice infected with lethal levels of *C. albicans* [1,2].

**Conclusions**

In this study, only availability of a genetic sequence that encodes the antigenic proteins is needed to produce oral vaccine based on molecular display technology. Because a vaccine produced using molecular display technology avoids the need for protein purification, this oral vaccine offers a promising alternative to the use of conventional vaccines for preventing a range of infectious diseases.

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**FEMS-1157**

**Fungal human pathogens**

**PROSPECTIVE STUDY ON ORAL CANDIDIAL INFECTION AFTER INTENSITY-MODULATED RADIATION THERAPY FOR NON-METASTATIC NASOPHARYNGEAL CARCINOMA: CORRELATION WITH THE RADIATION DOSE TO THE PAROTIDS**

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**Background**

Nasopharyngeal carcinoma (NPC) is endemic in Southern China. Intensity-modulated radiation therapy (IMRT) for NPC may damage the salivary glands and lead to xerostomia, providing an ideal environment for opportunistic candidial infections.

**Objectives**

We prospectively investigated the incidence and factors correlating with oral candidial infection in NPC patients treated with radical IMRT.

**Methods**

Forty five patients with non-metastatic NPC were prospectively recruited. Saliva and mouthrinse samples were saved at baseline and then two-weekly during IMRT. They all received IMRT with 70Gy in 33 to 35 fractions over 7 weeks concurrent with cisplatin chemotherapy. Besides the gross tumour and positive neck nodes, the parotids, submandibular and sublingual glands were contoured on Eclipse Treatment Planning System™ for radiation dose calculation. Fungal culture was performed on Sabouraud dextrose agar and CHROMagar™. In addition, API-32C AUX method and species-specific Taqman probes were used for specific identification of *Candida* species. Considerable number of patients i.e. 9 (20.0%) and 25 (55.6%) patients had clinical diagnosis of oral candidiasis and positive candidial culture respectively during IMRT. *C. parapsilosis* (24 patients, 53.5%) followed by *C. albicans* (18 patients, 40.0%) were most commonly found. Grade 2 and 3 xerostomia were noted in 13 (28.9%) and 32 (71.1%) patients respectively. Univariable and multivariable analysis



revealed that mean radiation dose to the parotids  $\geq 45\text{Gy}$  was the only factor ( $p=0.045$  and  $p=0.043$  respectively) correlating significantly with occurrence of oral candidial infection.

## **Conclusions**

Present study demonstrated that IMRT to the parotids may predispose to oral candidial infections which should be taken into account when managing NPC patients.

**FEMS-2601**

**Fungal human pathogens**

**RAPID DETECTION OF CYP51A-PROMOTER BASED VORICONAZOLE RESISTANCE IN ASPERGILLUS FUMIGATUS ISOLATES IN A HIGH INCIDENCE POPULATION**

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**Background**

Due to increased azole resistance, first line treatment with voriconazole of *Aspergillus fumigatus* infections in humans is increasingly difficult. Susceptibility testing is essential but culture based resistance determination takes up to two weeks. Resistance is predominately based on mutations in *cyp51A* and duplications of the promoter region (TR34 & TR46).

**Objectives**

To develop a rapid molecular method to screen for azole resistance in *A. fumigatus*.

**Methods**

Azole susceptibility profiles and *cyp51A* genotypes were determined in 105 isolates of 105 consecutive high-risk patients. A probe-based multiplex qPCR was designed and evaluated. This qPCR specifically amplifies the promoter region of *cyp51A* and uses a *cyp51A* control probe and probes for the TR34 and TR46 voriconazole resistance associated promoter duplications.

**Conclusions**

24/105 (22.9%) of the patient isolates had elevated voriconazole MICs. Sequencing of *cyp51A* showed that in 18/24 (75%) isolates the resistance was associated with mutations in the *cyp51A* ORF and had a tandem repeat duplication in the promoter region, identifying this as the main resistance mechanism. The qPCR detected 18 of the 18 isolates in which ORF mutations and promoter duplication were present. The 6 resistant isolates that lacked duplications in the promoter region, as well as all sensitive strains were not detected by the PCR. The probe-based multiplex qPCR delivers a rapid method for the identification of the majority of azole resistant *A. fumigatus* isolates, and offers a rapid pre-screen for detection of voriconazole resistance in a routine clinical routine setting.



**FEMS-2903**

**Cell-cell communication / Quorum sensing: inhibition as novel therapeutic tool**

**Quorum quenching acylases for reducing bacterial virulence**

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The observation that acylases from the NTN-hydrolase family can cleave the amide bond between acyl side chains and the homoserine lacton moiety of bacterial signalling molecules has raised novel options for interfering with quorum sensing and the onset of virulence. NTN-hydrolases that have initially become known by their paradigm representatives penicillin and cephalosporin acylase appear to be very abundantly encoded in bacterial genomes. Intriguingly the *Pseudomonas aeruginosa* genome contains not less than 4 NTN-hydrolases. The acylase PvdQ is extremely effective in quorum-quenching, hydrolysing bacterial N-acylhomoserine lactones at very low concentrations. Exogenously added PvdQ can reduce the virulence and we have determined its 3D structure. QuiP and HacB are two other acylases hydrolyzing a subset of the signalling molecules of Gram-negatives, C12-HSL and 3-oxo-C12-HSL. The hydrolytic activity of PvdQ is the highest for long-chain AHLs such as 3-oxo-C12-HSL and decreases with shorter acyl chain length, with hardly any activity for C8-HSL. A structure-based approach was applied to evolve this enzyme into an effective quorum quencher of the communication molecule (C8-HSL) of *Burkholderia* species, opportunistic pathogens commonly isolated together with *P. aeruginosa* from patients suffering from cystic fibrosis. Two point mutations in the active centre of PvdQ were found to increase the activity of this enzyme towards C8-HSL. The combination variant displayed high hydrolytic activity towards C8-HSL when compared to wild-type PvdQ. The effects could be strongly correlated in vivo, as exogenous addition of this mutant led to a significant decrease of autoinducers present in *Burkholderia cenocepacia* cultures, and effectively reduced its virulent phenotype.

**FEMS-0637**

**Cell-cell communication / Quorum sensing: inhibition as novel therapeutic tool**

**Chemical Interception of Bacterial Quorum Sensing: New Languages, New Outcomes**

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We are developing chemical tools that attenuate cell-cell communication pathways in bacteria. Many bacteria communicate using small organic molecules and peptides to monitor their population densities in a process called “quorum sensing.” At high cell densities, bacteria use this signaling network to switch from an isolated, nomadic existence to that of a multicellular community. This lifestyle switch is significant; only in groups will pathogenic bacteria turn on virulence pathways and grow into drug-impervious communities called biofilms that are the basis of myriad chronic infections. In turn, certain symbiotic bacteria will only initiate host-beneficial behaviors at high population densities. The molecular mechanisms of these processes are only now being delineated. Our research is broadly focused on the design and chemical synthesis of non-native ligands that can intercept quorum sensing and provide new insights into its role in host/microbe interactions. We have developed a series of efficient synthetic methods that provide us with straightforward access to these ligands. In addition, we have applied both quorum sensing antagonists and agonists *in vitro* and *in vivo* to investigate quorum sensing as an anti-infective target. Quorum sensing pathways in Gram-negative and Gram-positive bacteria have been our foci, including LuxI/LuxR-type signaling in *Pseudomonas aeruginosa* and agr-type signaling in *Staphylococcus aureus*. This presentation will introduce our research approach and highlight recent results.

**FEMS-1017**

**Cell-cell communication / Quorum sensing: inhibition as novel therapeutic tool**

**AHL ACYLASE DISRUPTS BIOFILM FORMATION OF NOSOCOMIAL PATHOGEN ACINETOBACTER NOSOCOMIALIS M2**

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**Background**

*Acinetobacter* is a Gram negative pathogen associated with nosocomial infection and multidrug resistance. Biofilm formation, as one of the most important virulence determinants in *Acinetobacter*, is regulated by quorum sensing. Disruption of biofilm formation represents an interesting target for antivirulence drug development. One particular species, namely *Acinetobacter nosocomialis* strain M2 [1] (previously known as *A. baumannii*) produces a wide range of quorum sensing signaling molecule with 3-OH-C12-HSL as the primary AHL.

**Objectives**

The aim of this study was to investigate the efficacy of quorum quenching acylase enzymes in reducing biofilm formation of *A. nosocomialis* M2. Three acylases with different preference of AHLs substrate were used, namely **PvdQ** [2] (prefer long chain AHLs, such as C10-C14 HSL), **PvdQ<sup>Lα146W,Fβ24Y</sup>** [3] (engineered PvdQ, active towards C8) and **QqaR** [4] (active towards C8-C14 HSL).

**Methods**

Static biofilm of *A. nosocomialis* M2 was grown in 96-well plate with or without addition of acylase. The production of biofilm was examined with crystal violet method.

**Conclusions**

The addition of AHL acylase was shown to disrupt biofilm production of *A. nosocomialis* M2, up to 50% reduction compare to control. Interestingly, PvdQ<sup>Lα146W,Fβ24Y</sup> and QqaR are the most effective in biofilm disruption.

**References**

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[3] *Proc Natl Acad Sci U S A* **2014**, 111, 1568–1573.

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FEMS-1581

Cell-cell communication / Quorum sensing: inhibition as novel therapeutic tool

## DEVELOPMENT OF QUORUM QUENCHING PEPTIDES TARGETING VIRR/VIRS SYSTEM OF CLOSTRIDIUM PERFRINGENS

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<sup>2</sup>Department of Bacteriology, University of Kanazawa, Kanazawa, Japan

### Background

*Clostridium perfringens* is an anaerobic gram-positive pathogen and causative agent for gas gangrene as well as necrotic enteritis. *C. perfringens* produces several extracellular enzymes and toxins, such as  $\alpha$ ,  $\theta$  and  $\kappa$  encoded by *plc*, *pfoA*, and *colA*, respectively. A two component regulatory system (TCS), VirR/VirS, controls production of these enzymes and toxins. Recently, we have identified a 5-membered macrocyclic oligopeptide (CLWFT) as an autoinducer (AIP<sub>Cp</sub>) of VirR/VirS TCS.

### Objectives

The VirR/VirS system is an attractive target for the development of anti-pathogenic chemotherapy of *C. perfringens*, because it orchestrates the quorum sensing cascade. To gain effective inhibitor to block the VirR/VirS signal transduction, we begin with drug design and development of peptides antagonist against AIP<sub>Cp</sub>.

### Methods

To develop antagonistic peptides targeting VirR/VirS, each amino acid residue of AIP<sub>Cp</sub> was substituted to different amino acids and their agonist activity, which induces *pfoA* transcription in *agrBD*-null mutant, as well as antagonist activity which inhibits *pfoA* transcription in wild type strain were examined.

### Conclusions

Alanine scanning suggests that whole structure of AIP is involved in agonist activity. Especially, Z-AIP<sub>Cp</sub>-W3A showed neither agonist nor antagonist activity, while AIP<sub>Cp</sub>-F4A solely showed weak but significant antagonist activity, suggesting that Trp-3 and Phe-4 are crucial for receptor binding and receptor activation, respectively. Interestingly, exchange of Trp-3 and Phe-4 yielded a potent antagonist with IC<sub>50</sub> = 0.4  $\mu$ M. Alternately, Z-AIP<sub>Cp</sub>-L2A-T5A also showed a potent antagonist activity of IC<sub>50</sub> = 0.3  $\mu$ M. High potential of these oligopeptides is suggested that these can be significantly used for therapeutic applications.



## NON-SOCIAL ADAPTATION DEFERS A TRAGEDY OF THE COMMONS IN *PSEUDOMONAS AERUGINOSA* QUORUM SENSING

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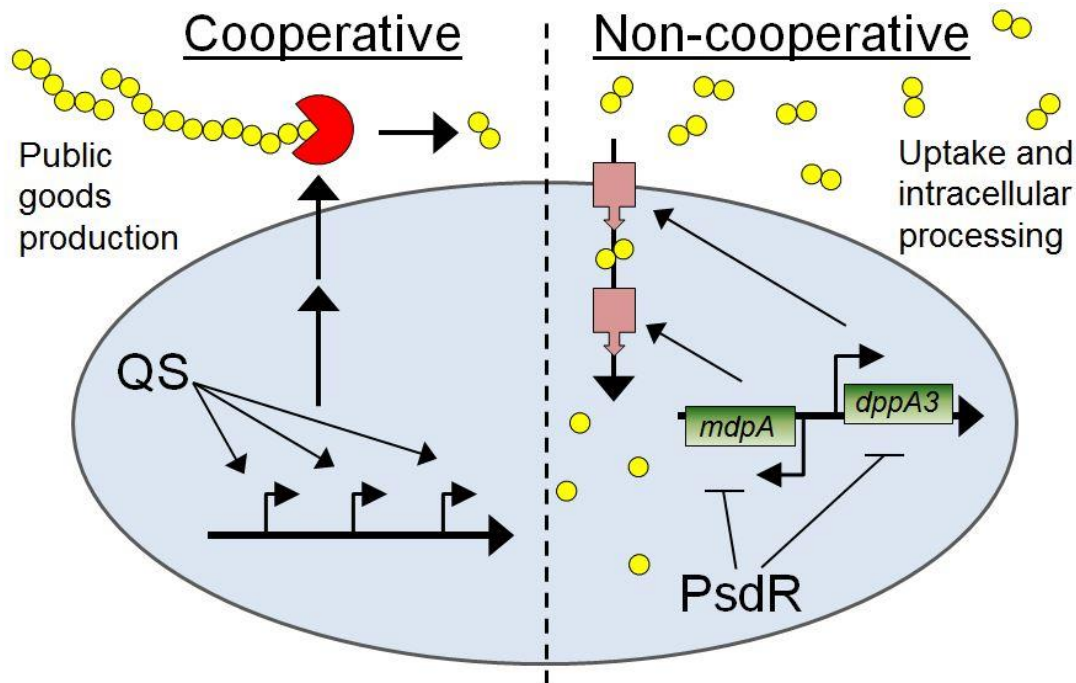
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### Background

In a process termed quorum sensing (QS), the opportunistic bacterial pathogen *Pseudomonas aeruginosa* uses diffusible signaling molecules to regulate the expression of numerous secreted factors or public goods that are shared within the population. But not all cells respond to QS signals. These social cheaters typically harbor a mutation in the QS receptor gene *lasR* and exploit the public goods produced by cooperators.

### Objectives

Here we show that non-social adaptation under growth conditions that require QS-dependent public goods increases tolerance to cheating and defers a tragedy of the commons.



### Methods

The underlying mutation is in the transcriptional repressor gene *psdR*. This mutation has no effect on public goods expression but instead increases individual fitness by derepressing growth-limiting intracellular metabolism. Even though *psdR* mutant populations remain susceptible to invasion by isogenic *psdR lasR* cheaters, they bear a lower cheater-load than do wild-type populations, and they are completely resistant

to invasion by *lasR* cheaters with functional *psdR*. Mutations in *psdR* also sustain growth near wild-type levels when paired with certain partial loss-of-function *lasR* mutations. Targeted sequencing of multiple evolved isolates revealed that mutations in *psdR* arise before mutations in *lasR*, and rapidly sweep through the population.

### **Conclusions**

Our results indicate that a QS-favoring environment can lead to adaptations in non-social, intracellular traits that increase the fitness of cooperating individuals and thereby contribute to population-wide maintenance of QS and associated cooperative behaviors.

FEMS-1394

Cell-cell communication / Quorum sensing: inhibition as novel therapeutic tool

## QUORUM SENSING-DEPENDENT REGULATION OF RHAMNOLIPIDS IN BURKHOLDERIA GLUMAE IS NUTRITIONALLY CONDITIONAL

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### Background

*Burkholderia glumae* is a phytopathogenic bacterium that uses the LuxR/LuxI-type TofI/TofR Quorum Sensing (QS) system to regulate various functions, including the major virulence determinants toxoflavin and flagella. *B. glumae* also releases surface-active rhamnolipids which, along with flagella, are required for the social behavior called swarming motility in *Pseudomonas aeruginosa* and *Burkholderia thailandensis*.

### Objectives

We investigated the role of rhamnolipids in swarming motility and the interplay between QS and nutrition-based regulation of rhamnolipids production in *B. glumae*.

### Methods

Swarming and swimming assays were carried out to test the motility of various mutants. Analyses of rhamnolipids and N-acyl-homoserine lactones were performed by LC-MS/MS. *rhlA* expression in wild-type and QS-defective *toft* strains was monitored using a *lux* reporter fusion.

### Conclusions

We show that QS positively regulates the production of rhamnolipids in *B. glumae* and these are also necessary for swarming motility in this species. Unexpectedly, the C<sub>8</sub>-HSL-negative *toft* mutant, in contrast with growth in a minimal medium, regains the ability to produce rhamnolipids in a complex nutrient broth medium. Moreover, decreasing nutrient concentrations leads to a major induction in *rhlA* expression in the wild-type strain. However, *rhlA* expression in the *toft* mutant is induced to a lesser extent by nutrient limitations. Under nutrient-limited conditions, QS mediates rhamnolipids biosynthesis, presumably to promote swarming motility. These results indicate that, in contrast with the situation in *P. aeruginosa*, QS regulation of rhamnolipids in *B. glumae* is dispensable and nutritionally conditional.

**FEMS-3153**

**Jorgen Lehmann and Gerhard Domagk event on *Mycobacterium tuberculosis*:  
New insights into the pathogenesis of MTB impacting on the host's immune  
response**

**TB vaccines; on the role of Th1, memory and immunity in the lung?**

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**TB vaccines; on the role of Th1, memory and immunity in the lung**

*Mycobacterium tuberculosis* and humans have co-evolved since the most early human origin and this has allowed the pathogen to adapt and develop a refined set of countermeasures to the human immune response during infection. The result is a pathogen that is rarely cleared by the natural immune response but instead establishes a long-term chronic infection. Although IFN- $\gamma$  is clearly insufficient for the control of TB in individuals that develop disease, most TB vaccine research have still been focused on immuno-dominant antigens administered in delivery systems that promote strong Th1 responses. Recent breakthroughs in our understanding of requirement for immunological memory and the recruitment and expression of immunity to TB in the lung now provide a framework for reconsidering that strategy.

I will discuss recent data from mouse and non-human primate models of vaccine promoted protective T cells. Our data suggest that instead of boosting Th1 and IFN- $\gamma$ , supplementing BCG with T<sub>CM</sub> that are insufficiently induced by BCG may be a feasible avenue towards TB vaccines that efficiently protect the TB infected lung. The data from animal models will be compared to the immune profile obtained with the H1/H56 vaccine in ongoing clinical trials.

**FEMS-2783**

**Jorgen Lehmann and Gerhard Domagk event on Mycobacterium tuberculosis:  
New insights into the pathogenesis of MTB impacting on the host's immune  
response**

## **THE ROLE OF THIOLS IN PROTECTION AGAINST OXIDATIVE STRESS IN MYCOBACTERIA**

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### **Background**

Low molecular weight thiols maintain the reducing environment in cells and are responsible for protecting cells against oxidants and toxins. In mycobacteria such as *Mycobacterium tuberculosis*, the major low molecular weight thiol is mycothiol (MSH), which has similar protective functions as glutathione (GSH), the LMW thiol present in eukaryotes and gram-negative bacteria. Other LMW thiols present in mycobacteria include ergothioneine (ESH), whose sulfur atom is most stable in solution in the thione form, and thioredoxins, small redox proteins containing a dithiol-disulfide active site. The relative contributions of these LMW thiols in protecting mycobacteria against oxidative stress is not clear.

### **Objectives**

The objective of the study was to characterize the mutants to evaluate their role in oxidative stress defense.

### **Methods**

*Mycobacterium smegmatis* mutants lacking MSH, ESH, and disrupted in thioredoxins and thiol peroxidases were evaluated for sensitivity to oxidative stress. Quantitative real-time PCR was also performed to determine the expression of the thiol associated genes. Also, the levels of thiols, protein carbonylation and lipid oxidation were determined for the mutants.

### **Conclusions**

A majority of the mutants were sensitive to oxidative stress and a significant increase in expression of genes involved in thiol biosynthesis and genes coding for thiol peroxidases occurred on oxidant exposure. Protein carbonylation and lipid oxidation levels were higher in MSH mutants as compared to mutants lacking ESH and thiol peroxidases. Our data suggests that MSH rather than ESH plays an important thiol in protection against oxidative stress and there is redundancy in protection against oxidative stress.

**FEMS-1369**

**Jorgen Lehmann and Gerhard Domagk event on Mycobacterium tuberculosis:  
New insights into the pathogenesis of MTB impacting on the host's immune  
response**

**DEVR DEPENDENT SYNCHRONIZATION OF MYCOBACTERIAL GROWTH AND  
ITS EFFECT ON MYCOBACTERIOPHAGE DEVELOPMENT**

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**Background**

Hypoxic conditions are known to induce a state of dormancy in mycobacterial species. To what extent phage growth is affected in mycobacterial cells exposed to hypoxia is not known.

**Objectives**

We attempted to investigate this by studying the growth properties of mycobacteriophage D29 in *Mycobacterium smegmatis* hosts exposed to hypoxia.

**Methods**

*M. smegmatis* cells were subjected to hypoxia either by limiting O<sub>2</sub> supply (Wayne Model) or by treatment with vitamin C, an O<sub>2</sub> scavenger. Treated cells were then infected with D29 and phage growth was monitored by plaque assays.

**Conclusions**

1. The results indicate that the phage grows poorly under hypoxia but its proliferation is enhanced during the recovery phase.
2. Hypoxia sensors DevR-DevS/DosT may have a role to play in the process as DevR deletion mutant did not support the observed increase in phage growth.
3. The growth properties of the *wt* and *devR* *M. smegmatis* strains were compared in presence and absence of O<sub>2</sub> and was observed that the growth of *wt* cells was retarded by hypoxic conditions but the *devR* strain was unaffected. So, DevR plays an active role in retarding the growth of Mycobacterial cells when they are exposed to hypoxia.
4. Following the return to normoxia the retarded cells were found to resume growth in synchrony.
5. Synchronous growth was found to benefit phage development because most of the cells were in the same metabolic state. D29 thus indirectly uses DevR-DevS signalling system to its own advantage when their hosts were exposed to hypoxia.

**FEMS-0824**

**Jorgen Lehmann and Gerhard Domagk event on Mycobacterium tuberculosis:  
New insights into the pathogenesis of MTB impacting on the host's immune  
response**

**MSMEG\_2433 OF MYCOBACTERIUM SMEGMATIS EXHIBITS BOTH DD-CARBOXYPEPTIDASE AND BETA-LACTAMASE ACTIVITIES**

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**Background**

DD-carboxypeptidases (DD-CPases) are low-molecular mass (LMM) penicillin-binding proteins (PBPs), mainly involved in the peptidoglycan remodelling, but little is known about the DD-CPases of mycobacteria. In this study, a putative DD-CPase of *Mycobacterium smegmatis*, MSMEG\_2433 has been characterized.

**Objectives**

- \*Physiological characterization of the membrane bound form of MSMEG\_2433
- \*Biochemical characterization of the soluble form of MSMEG\_2433

**Methods**

The gene for the membrane-bound form of *MSMEG\_2433* was cloned and expressed in *E. coli* in its active form as revealed by its ability to bind to the Bocillin-FL (fluorescent penicillin). Interestingly, *in vivo* expression of MSMEG\_2433 could restore the cell-shape oddities of the septuple PBP mutant of *E. coli*, which was a prominent physiological character of DD-CPases. Moreover, expression of MSMEG\_2433 *in trans*, elevated the beta-lactam resistance in the PBP deletion mutants ( $\Delta dacAdacC$ ) of *E. coli* that strengthened its physiology as a DD-CPase. To confirm the biochemical reason behind such physiological behaviours, soluble form of MSMEG\_2433 (sMSMEG\_2433) was created, expressed and purified. In agreement to the observed physiological phenomena, the sMSMEG\_2433 exhibited the DD-CPase activity against artificial and peptidoglycan-mimetic DD-CPase substrate. The enzymatic analyses of MSMEG\_2433 revealed the efficient deacylation for beta-lactam substrates, which is unique for beta-lactamases. In addition, *in silico* molecular analyses predicted the presence of an active-site that favours DD-CPase activity of MSMEG\_2433 and an omega-loop like region that determines its beta-lactamase activity.

**Conclusions**

Based on the *in vitro*, *in vivo* and *in silico* studies we conclude that MSMEG\_2433 is a dual enzyme, possessing both DD-CPase and beta-lactamase activities.





**FEMS-2852**

**The diversity of bacterial nanomachines**

**Unfolded protein transport across membranes: The Injectisome in Action**

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We are focusing on two fundamental aspects in biology (1) assembly of multicomponent systems into functional nanomachines and (2) transport of proteins across membranes. Both are found in type three secretion systems (T3SS), which are required of bacterial secretion of proteins into the host that subvert the host antibacterial response in order to promote infection. These systems utilize a 3.5 MDa syringe-like, membrane embedded “injectisome” containing an ~800 Å long needle complex thought to cross multiple membranes to connect the intracellular compartments of the bacteria and the host. The striking resemblance of the injectisome to a bona fide syringe, revealed by electron microscopy has led to the hypothesis that bacterial proteins are secreted through the needle complex into the host. I will discuss how and where substrates are passed through the T3SS and across two membranes by visualizing injectisomes in action and in situ.

([www.marlovitslab.org](http://www.marlovitslab.org))

**FEMS-2788**

**The diversity of bacterial nanomachines**

**A new class of rotary complexes promote motility and sporulation in bacteria**

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Bacteria move over solid surfaces by so-called gliding motility, a process whereby the bacterium moves smoothly along its long axis in absence of extracellular organelles. Due to the small size of the bacterial cell the gliding mechanism has long remained enigmatic. In recent years, we identified the *Myxococcus xanthus* motility engine and showed that it localizes to discrete sites along the cell long axis. However, the exact propulsion mechanism remained undetermined. In this presentation, I will present the motility machinery and recent work combining genetics, biophysics and modeling that allowed us to elucidate the motility mechanism.

**FEMS-1564**

**The diversity of bacterial nanomachines**

**BACTERIAL TWITCHING MOTILITY IS COORDINATED BY A TWO-DIMENSIONAL TUG-OF-WAR WITH DIRECTIONAL MEMORY**

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**Background**

Many bacterial species possess appendages that allow them to move while staying attached to the surface. One of the most ubiquitous cell appendages that mediate movement is the type IV pilus system.

Type IV pilus dependent movement has been known as 'twitching motility' for a long time, but only recently we were able to demonstrate experimentally that *Neisseria gonorrhoeae* moves with directional persistence and proposed that its multiple pili are coordinated through a tug-of-war mechanism. [1]

**Objectives**

We addressed the question whether the pilus dynamics was coordinated mechanically and thereby promoting persistent movement, a mechanism that has been shown to be efficient in other processes.

**Methods**

We used a combined theoretical and experimental approach to test this hypothesis. Using laser tweezers we measured detachment rates and pilus retraction speeds at the level of single pili. Using this quantitative data we build a tug-of-war model.

**Conclusions**

Unexpectedly, we found that the two-dimensional tug-of-war is less efficient in generating persistent motion than the one-dimensional tug-of-war studied previously in the context of cytoskeletal transport. [2] The systematic comparison of the patterns of motility with the corresponding experimental data showed that a form of directional memory is required to explain experimental observations. Experimentally, we confirmed memory in the form of bursts of pilus retraction.

Overall, type IV pili are essential for two opposing phenotypes in *N. gonorrhoeae*, namely motility and clustering into microcolonies and biofilms. Directional persistence is likely to bias the behaviour towards motility.



**FEMS-1407**

**The diversity of bacterial nanomachines**

**MICROCYSTIS AERUGINOSA NANOWIRES: AN ELECTRIFYING TALE**

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**Background**

Microorganisms are known to produce extracellular, proteinaceous nanofilaments called type IV pili (TFP) which are known to be involved in different functions like twitching motility, adhesion, biofilm formation etc.. These TFP from *Geobacter sulfurreducens* were found to be electrically conductive and termed as microbial nanowires.

**Objectives**

As early studies on microbial nanowires focussed on anaerobic microorganisms, this study was undertaken to identify, optimize and structurally characterize microbial nanowires in *Microcystis aeruginosa*.

**Methods**

For electrical characterization, conductive atomic force microscopy (CAFM) was used while for structural characterization, transmission electron microscopy (TEM) and *in-silico* methods were employed. For optimal production of pili, cells were subjected to different stressful culture conditions i.e. carbon source limitation and high light intensity and their piliation level were qualitatively checked using TEM.

**Conclusions**

TFP in *M. aeruginosa* was observed to be 8.5-11nm in diameter and 2μ to several μ long. They seem to be either composed of two distinct subfilaments or may be hollow cylinders. The piliation level in *M. aeruginosa* was sensitive to the culture conditions used. CAFM study showed that TFP in *M. aeruginosa* are electrically conductive in nature and can act as a microbial nanowire. *in-silico* studies suggested that aromatic amino acids may be involved in electron transfer through these TFP. The role of such nanowires in toxic bloom formation needs to be investigated further. This study significantly increases our understanding about aerobic cyanobacterial nanowires and also gives impetus for further research in this field and potential environmental applications.

**FEMS-1707**

**The diversity of bacterial nanomachines**

**THE BACTERIAL FLAGELLUM OF SALMONELLA: PROTEIN EXPORT MECHANISMS OF A MACROMOLECULAR MACHINE**

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**Background**

*Salmonella* swims through liquid environments by rotating a helical organelle, the flagellum. This flagellum is closely related to virulence-associated injectisome systems. A type-III protein secretion (T3S) apparatus is utilized to secrete building blocks of the flagellum, structural subunits of the injectisome complex, as well as virulence effectors into host cells.

**Objectives**

The T3S apparatus utilizes both the energy of the proton motive force (PMF) and ATP hydrolysis to energize substrate unfolding and translocation. However, the role of the T3S ATPase and other export apparatus components remained unclear.

**Methods**

We used bacterial genetics, fluorescent microscopy and reporter secretion assays to probe the function of the T3S apparatus *in vivo*.

**Conclusions**

We report formation of flagella in the absence of T3S ATPase activity by mutations that increased the PMF and flagellar substrate levels. We additionally show that increased PMF bypassed the requirement of the Spi1 virulence-associated T3S ATPase for secretion. We thus demonstrate that the actual export process is energized by the means of the proton motive force and does not require ATP hydrolysis.

Finally, we performed a genetic screen to dissect the minimally essential components of the flagellar T3S apparatus. We show that most integral-membrane components are essential and all cytoplasmic components are dispensable for export, emphasizing the importance of the core inner-membrane export apparatus components for the function of type-III secretion systems.

In summary, our data support a role for T3S ATPases in enhancing secretion efficiency under limited secretion substrate concentrations and reveal the essential core components of the T3S apparatus.

## **FEMS-1990**

### **The diversity of bacterial nanomachines**

#### **THE STRUCTURE OF THE TYPE SIX SECRETION SYSTEM CONTRACTILE SHEATH SOLVED BY CRYO ELECTRON MICROSCOPY**

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#### **Background**

Bacteria use rapid contraction of a long sheath of the Type VI secretion system to deliver effectors into a target cell.

#### **Objectives**

The mechanism of contraction is still unclear, mostly due to a lack of a high-resolution structure of the sheath.

#### **Methods**

We used cryo-electron microscopy and helical reconstruction to solve an atomic resolution structure of a native *Vibrio cholerae* sheath. Fluorescence microscopy and bacterial competition assays confirm central findings.

#### **Conclusions**

The localization of the outer domain of the sheath facilitates unfolding by ClpV. Structural alignments with phage sheaths indicate a conserved mechanism of interaction. In the innermost layer, a 4-stranded  $\beta$ -sheet from three proteins links the protomers in the same strand as well as neighboring strands. This  $\beta$ -sheet is crucial for polymerization and stability.

**FEMS-2552**

## **Syntrophic interactions in anaerobic communities**

### **New insights in syntrophy in methanogenic communities**

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### **New insights in syntrophy in methanogenic communities**

In methanogenic environments, syntrophic communities form the core of the organic acid degrading population. Organic acids are only efficiently converted if the syntrophs maintain a close relationship, driven by interspecies electron transfer. The eco-physiology of syntrophs is poorly understood as only a few syntrophs have been thoroughly characterized. To clarify their eco-physiology, it is important to study syntrophs *in situ* but also understand the biochemical mechanisms they have to adapt their metabolism to changing environmental conditions.

Based on genome analysis of our model syntrophic propionate-degrading community *Syntrophobacter fumaroxidans* and *Methanospirillum hungatei* and expression of genes involved in interspecies electron transfer we revealed mechanisms that enable the coculture to adapt<sup>1</sup>.

Single-cell microbiology enables microbiologists to observe who does what, when, where and next to whom<sup>2</sup>. Single cells taken from the environment can be identified and their genome sequenced. Single-cell based gene expression analysis could be a valuable tool in interpreting gene functions and microbial metabolism in various syntrophic communities and reveal genes and gene clusters that are involved in syntrophy<sup>3</sup>.

This presentation will discuss syntrophy and methanogenic syntrophic communities and emphasize on recent developments and discoveries.

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- 3) Qi Z, G Pei, L Chen, WW Zhang (2014) Single-cell analysis reveals gene-expression heterogeneity in syntrophic dual-culture of *Desulfovibrio vulgaris* with *Methanosarcina barkeri* *Scientific reports* Sci Rep 4: 7478; DOI: 10.1038/srep07478



**FEMS-2845**

**Syntrophic interactions in anaerobic communities**

**Studying microbial interactions using electrochemical tools**

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**Microbial collaboration for effective electron transfer**

Research on extracellular electron transfer has accelerated in the past ten years. Whereas the process itself was described in 1986, only in the 2000s major interest emerged leading to the discovery of direct electron transfer to and from electrodes, and more recently the discovery that pili or pilus-like appendages are involved in long range electron transfer. The electrical interactions are not only occurring between a microorganism and an electrode or mineral, it is now clear that microorganisms interact among each other via electrons. It is likely that they do so to link their overall respiration, settling charge balances via solid state transfer rather than relying on diffusive processes. Taking this information on board, earlier findings on the microbial ecology of electroactive biofilms make more sense and it appears that in anaerobic environments the physiology related to electron transfer needs to be included when considering aspects such as syntrophic interactions. In my presentation, I will give an overview of the findings by many researchers worldwide, and try to assess how this impacts particular applications such as microbial electrosynthesis, microbial electrochemical systems and anaerobic digestion processes.

**FEMS-1988**

**Syntrophic interactions in anaerobic communities**

**PROTEOMICS OF SYNTROPHOMONAS ZEHNDERI AND METHANOBACTERIUM FORMICICUM GROWING ON LONG-CHAIN FATTY ACIDS**

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**Background**

Conversion of long-chain fatty acids (LCFA) in anaerobic digesters relies on syntrophic relationship between acetogenic bacteria and methanogenic archaea. Conversion of unsaturated- and saturated-LCFA has been previously shown by a co-culture of *Syntrophomonas zehnderi* and *Methanobacterium formicum*. Degradation of unsaturated-LCFA is rare among *Syntrophomonas* species; the best studied fatty-acid oxidizer, *S. wolfei*, can only grow on saturated-LCFA.

**Objectives**

Major differences are expected in the pathways and enzymes involved in the degradation of unsaturated-LCFA. In this work we used proteogenomic approach to study these differences.

**Methods**

A draft genome of *S. zehnderi* was obtained by IlluminaHiSeq sequencing. Genomes of *S. zehnderi* and *S. wolfei* (available at NCBI) were compared. *S. zehnderi* and *M. formicum* co-cultures grown on oleate (unsaturated LCFA, C18:1) and on stearate (saturated LCFA, C18:0) were further studied using a proteomics approach.

**Conclusions**

Genomic comparison of *S. zehnderi* and *S. wolfei* revealed approximately 900 different proteins and 1200 common proteins. In the genome of *S. zehnderi*, two replicates of the unsaturated acyl-CoA dehydrogenase genes were identified, one of which differs considerably from the acyl-CoA gene found in *S. wolfei*. Proteomic analysis of *S. zehnderi* and *M. formicum* co-cultures revealed high expression levels of proteins related to the  $\beta$ -oxidation of LCFA (up to 30% of total proteins identified). Different protein expression levels were observed during the degradation of oleate (44% unique proteins) and stearate (23% unique proteins). In addition, proteins involved in electron transfer were highly expressed, including electron transfer flavoproteins, ATP synthases and a number of hydrogenases and formate dehydrogenases.

**FEMS-2488**

**Syntrophic interactions in anaerobic communities**

**SYNTROPHIC COCULTURE OF GEOBACTER SULFURREDUCTENS AND  
SYNTROPHOBACTER FUMAROXYDANS GROWING WITH PROPIONATE AND  
Fe(III)**

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**Background**

The Fe(III)-reducing *G. sulfurreducens* is restricted to simple organic substrates like acetate as the electron donor and unable to metabolise propionate. *S. fumaroxidans* on the other hand cannot use Fe(III) as electron acceptor.

**Objectives**

Syntrophic coculture of the electroactive bacterium *Geobacter sulfurreducens* and the propionate oxidising bacterium *Syntrophobacter fumaroxidans* with propionate and Fe(III) were constructed.

**Methods**

*G. sulfurreducens* was first grown in pure culture with single and different combinations of the electron donors of acetate, formate and hydrogen as the products of propionate oxidation by *S. fumaroxidans*. All single electron donors supported the growth of *G. sulfurreducens* where  $6.44 \pm 0.02$ ,  $2.43 \pm 0.01$  and  $1.63 \pm 0.41$  mole of Fe(II) was produced per mole of acetate, formate and hydrogen, respectively. When different combination of electron donors applied, no acetate consumption was noted in the presence of formate. Moreover, in the presence of hydrogen, reduced amount of acetate/formate was needed for Fe(III) reduction compared with similar conditions without hydrogen. Next, our coculture results showed for the first time the successful growth of these two bacteria with propionate and Fe(III) in batch cultures, where neither of bacteria was independently capable of such growth.

**Conclusions**

The exceptionally high ratio of Fe(II) production per propionate consumption ( $17.3 \pm 2.52$ ) showed high potential of the applied coculture for iron reduction using propionate as the electron donor. Further molecular and microscopic studies are underway to illustrate the spatial structure of the coculture and the abundance of the two partner bacteria.



**FEMS-0669**

## **Syntrophic interactions in anaerobic communities**

### **SYNTROPHIC ETHANOL OXIDATION - A LONG WAY TO A CONVINCING SOLUTION**

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#### **Background**

Methanogenic conversion of ethanol to acetate plus methane has been described by Wolin et al. in 1967 as the first case of syntrophic cooperation between two species in methanogenic degradation. In the co-culture "*Methanobacillus omelianskii*", the so-called S-organism cooperated with a hydrogen-oxidizing methanogen. The oxidation pathway as conceptualized with this culture included alcohol dehydrogenase, acetylating acetaldehyde dehydrogenase, and subsequent ATP formation via phosphotransacetylase and acetate kinase. This concept has been confirmed later with *Pelobacter acetylenicus* and other *Pelobacter* species .

#### **Objectives**

*Pelobacter spp.* have to invest part of their ATP gain into a reversed electron transport to release electrons as hydrogen or formate. The overall energy balance leaves less than one ATP per reaction run for these bacteria. We wanted to elucidate the mechanism of electron flow from ethanol to hydrogen.

#### **Methods**

Studies included enzyme assays in cell-free extracts as well as genomic and proteomic analyses.

#### **Conclusions**

Genomic studies indicated that *Pelobacter carbinolicus* contains a comproportionating hydrogenase oxidizing NADH and ferredoxin simultaneously. Methanogenic ethanol-oxidizing cocultures maintained formate and hydrogen levels equivalent to a standard redox potential of  $E^0 = -360$  mV. Combined with further genomic information, our results provide a convincing concept for electron flow and ATP synthesis in syntrophically ethanol-oxidizing *Pelobacter* species.

**FEMS-2529**

**Syntrophic interactions in anaerobic communities**

**MICROBIAL SYSTEMS ECOLOGY: FROM DEFINING NICHES TO  
RECONSTRUCTING MATTER FLOWS THROUGH MICROBIAL CONSORTIA**

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**Background**

Mixed microbial communities are complex and dynamic systems. Integrated omics (combined metagenomics, metatranscriptomics, metaproteomics and metabolomics) are currently gaining momentum for detailed descriptions of community structure, function and dynamics *in situ*. These top-down analyses also allow inference of fundamental ecological properties of constituent populations and allow a description of the flow of matter through these systems.

**Objectives**

To define niche breadths of constituent populations and to infer the flow of matter through a mixed microbial community from integrated omics data.

**Methods**

We have developed an integrative workflow comprising wet- and dry-lab methodologies that enables systematic measurements of microbial communities, and the integration and analysis of the resulting “multi-meta-omic” data including in the context of reconstructed metabolic networks. Here we apply this methodological framework to our model microbial communities (lipid accumulating microbial consortia from a biological wastewater treatment tank) sampled over space and time.

**Conclusions**

By resolving multi-omic data at the population-level, we are able to infer lifestyle strategies (generalists versus specialists) of the respective constituent populations. More specifically, through our analyses, we have uncovered patterns which suggest that, under rapidly fluctuating environmental conditions, the dominance of a microbial generalist is linked to finely tuned resource usage, and that genetic variation within generalist populations is constrained. Furthermore, by reconstructing population-resolved metabolic networks and interfacing there, we are able to partially reconstruct the flow of matter through the community. These results bring us closer to being able

to infer key ecological characteristics of constituent microbial populations as well as matter and ultimately energy flows through communities.

## **FEMS-2545**

### **Secretion in Bacteria**

#### **Mechanisms of Bacterial Secretion**

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#### **Mechanisms of Bacterial Secretion**

**Gabriel Waksman**<sup>1</sup>

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Type IV secretion (T4S) systems are molecular machines used for the transport of macromolecules across the bacterial cell envelope. T4S systems are highly versatile. Conjugative T4S systems translocate DNA from a donor to a recipient bacterium and contribute to bacterial genome plasticity, spread of antibiotic resistance or other virulence trait among bacterial pathogens. In some bacteria such as *Helicobacter pylori* (Cag PI), *Brucella suis* (VirB/D), or *Legionella pneumophila* (Dot, Icm), T4S systems are directly involved in pathogenicity as they mediate the secretion of virulence factors (DNA or toxins) into host cells. The archetypal T4S system, the VirB/D system, was defined in *Agrobacterium tumefaciens* where it is naturally responsible for the delivery of the T-DNA to the plant host-cell. The *A. tumefaciens* VirB/D system comprises 12 proteins (VirB1 to 11 and VirD4). Recently, structures of large complexes formed by several of these proteins have become available shedding unprecedented light on T4S system secretion mechanism.

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## **FEMS-0605**

### **Microbial responses to illumination**

#### **On the role of photosensory receptors in (chemo) trophic bacteria**

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Oxygenic photosynthesis and its applications in (energy) biotechnology will have to have a key role in a sustainable future. It is therefore significant that this process can be straightforwardly engineered in oxygenic phototrophic organisms, such as cyanobacteria, using the tools of synthetic biology. This has led to the emergence of cell factories that catalyze the (sun)light-driven conversion of CO<sub>2</sub> and water into products like ethanol, butanol, or other bio-fuels, commodity chemicals like lactic acid, a bio-plastic precursor, and even artificial sweeteners and flavor compounds. Only oxygen is produced as a by-product. For successful application of this approach it is of key importance to optimize such cell factories to maximal efficiency. This holds for their light-harvesting capabilities, e.g. under circadian illumination in large-scale photo-bioreactors, but equally well for the 'dark' reactions of photosynthesis, i.e. the conversion of CO<sub>2</sub>, NADPH and ATP into a (biofuel) product.

After an introduction of the versatility of this approach, an analysis based on metabolic control theory is presented of the amount of the enzymes catalyzing the heterologous metabolic pathway for product formation with which such cyanobacterial cell factories have to be equipped for maximal rates of product formation. For this engineered L-lactic acid producing *Synechocystis* sp. PCC6803 strains are used to identify the relation between actual production rate and expressed enzyme capacity. The analysis shows that engineered cell factories (e.g. for L-lactic acid) require high capacity of the heterologous product-forming pathway, before control in other parts of the cell factory can be detected and relief of limitation in those other parts becomes relevant.

#### **Reference**

\* Angermayr et al. (2014) *Biotechnol. Biofuels* 7: 99.

**FEMS-2307**

**Microbial responses to illumination**

**Photoprotection in cyanobacteria and the photoactive Orange Carotenoid Protein**

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The water-soluble orange carotenoid protein (OCP), a cyanobacterial protein of 35kDa which binds a single keto-carotenoid was first described and isolated by Holt and Krogman in 1981. During long time its role was unknown. In 2006 we demonstrated that OCP is essential in photoprotection in cyanobacteria. Cyanobacteria protect themselves from intense sunlight by dissipating excess absorbed energy as heat at the level of their extramembranal antenna, the phycobilisomes. This decreases the formation of dangerous species of oxygen and diminishes photodamage. The process is induced by the absorbance of blue-green light by the OCP, which acts as the light intensity sensor and the excitation energy quencher and is a new member of the family of photoactive proteins. It is the first photoactive protein containing a carotenoid as the photoactive chromophore and its photocycle is completely different from those of other photoactive proteins. The absorbance of blue-green light by the OCP induces structural changes in the carotenoid and the protein, converting its dark stable orange form (OCP<sup>o</sup>) into a relatively unstable active red form (OCP<sup>r</sup>). OCP<sup>r</sup> form is essential for the induction of the photoprotective mechanism. Only OCP<sup>r</sup> is able to interact with phycobilisomes and to quench excess energy. A second protein, the Fluorescence Recovery Protein, is needed to detach the OCP<sup>r</sup> from phycobilisomes and converted to the inactive OCP<sup>o</sup> form. All the known details of this mechanism will be described in the conference. We have recently demonstrated that OCP has another essential role in the cells: it's a very good singlet oxygen quencher. Thus, OCP has dual and complementary photoprotective action as energy quencher and as singlet oxygen quencher.

**FEMS-3151**

**Microbial responses to illumination**

**Signaling by Phytochrome, A Knotted Bacterial Photoreceptor**

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Phytochrome is a multidomain red-light photoreceptor that allows cells to respond to the quality and intensity of incoming light. Bacterial phytochromes adopt a deep figure-of-eight knotted polypeptide. They bind the linear tetrapyrrole biliverdin covalently via a thioether link to cysteine. Photon absorption causes a rotation about the C15=C16 double bond of this chromophore, leading to signaling through the conserved PHY domain to a modular output domain. To advance our research goals of understanding the structure/function relationships of bacterial phytochromes, we are studying the biochemistry and structural biology of the signal transduction pathway of the bacterial phytochromes of *Deinococcus radiodurans* and *Ramlibacter tatouinensis*. Each contains a histidine kinase domain, and each is predicted to be a light-regulated enzyme that phosphorylates across a homodimeric interface and subsequently transfers phosphate to a response regulator protein. We have established a robust *in vitro* kinase assay and have shown that *R. tatouinensis* bacterial phytochrome is an autokinase that interacts specifically with at least one cognate single domain response regulator (Brr). Kinase activity is markedly lower under red light. We have also refined the X-ray crystal structure of the Brr protein. A C-terminal extension of the canonical response regulator fold creates a unique dimer interface not seen outside the phytochrome-associated response regulators. We are engaged in determining the functional consequences of the knotted phytochrome topology and the oligomeric response regulator. We imagine both could limit motions of phytochrome domains upon illumination to steer signal transduction along a productive trajectory.

**FEMS-2865**

**The human virome: new tools and concepts**

**Increasing the knowledge of the human virome at homeostasis and disease by deep sequencing of clinical samples**

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The composition, interindividual and temporal variability of the human virome are not precisely known but new questions arise in the context of the rapidly increasing knowledge regarding its composition and function. Its impact on human health has received less attention than that of the bacterial microbiome, but is likely to be as important, both in homeostasis and disease. In fact, it is increasingly apparent that viral pathogens are only the salient members of a larger group of viruses associated with humans that are not directly linked to disease, analogous to the bacterial component of the microbiome. In particular, viruses abound at the body interfaces with the external environment, on the skin and in the mucosa. In contrast to this, the presence of viruses in the blood and organ parenchyma of healthy people is rather the exception than the rule, although a limited number of viral species establish persistent, albeit mostly silent, systemic infections, more often in immunocompromised individuals. With the ever-extending use of next generation, rapid progress on its impact on human health is to be expected in the coming years including the identification of viruses with roles in chronic diseases. We will show how deep sequencing of human clinical cases samples reveals, rather than new viral species, an unexpected role of known viruses in human diseases particularly in immunocompromised patients. On the other hand, whilst cross transmission from animals is preeminent in emerging viral diseases, close or identical new viral species are found in human and animals at homeostasis, which suggests more circulation between species than anticipated.

## **FEMS-3140**

### **The human virome: new tools and concepts**

#### **How to study phages in natural communities: tools, concepts and challenges**

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Microbes drive the biogeochemical cycles that fuel the planet, and their viruses play important roles ranging from mortality and nutrient cycling to gene transfer in environments ranging from the oceans and soils to industrial fermenters and humans. However, our inability to “see” viruses in the environment challenges our broader and quantitative understanding of viral roles in ecosystem processes. My lab specializes in developing new experimental and informatic approaches to better “see” and interpret the diversity of wild viruses across myriad data types and environments. Together these methods link viruses to their hosts *en masse*, provide both single-cell and population perspectives on virus-host interactions, or help elucidate Viral Dark Matter that dominates surveys of viruses in nature. In natural communities, we have mapped much of the global virome, shown that viral genome sequence space is clustered (into ‘populations’ formally delineated as species), and provided hypotheses on the role of viral metabolic reprogramming in modulating ecosystem function. This ability to now ‘count’ biologically meaningful units for viruses in nature – both genes and ‘organisms’ – opens watershed possibilities for applying ecological and evolutionary theory and developing predictive modeling across diverse ecosystems. Beyond the oceans, microbes are emerging as major players in terrestrial and atmospheric biogeochemical and energy cycling, as well as many diseases humans. Viral roles in modulating these microbial impacts are virtually unstudied, but likely to be large. To this end, my lab is now optimizing and applying these novel ocean-derived experimental and ‘big data’ analytical approaches to viral communities across diverse environments including sediments, the human lung and gut, and climate-sensitive thawing permafrosts, with aspirations to explore roles of viruses in the atmosphere and the Built Environment.

**FEMS-2893**

**The human virome: new tools and concepts**

**Human and animal viruses identified through metagenomics: pathogens, commensals, or contaminants?**

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Viral metagenomics is the unbiased amplification and sequencing of viral particles-associated nucleic acids and their classification using sequence similarity to known viruses. The research and diagnostic opportunities provided by this approach include the development of universal and sensitive virus identification tools and viral pathogen discovery. Our methodology and bioinformatics pipeline, together with their limitations, will be briefly described. Examples of virus and viral DNA contamination and the detection within mammalian samples of viral sequences originating from diverse cellular hosts will be shown. New viral genomes from human and animal samples with unexplained diseases of suspected infectious origins will be described together with the exp performed or required to demonstrate their pathogenicity. Some of these newly described viruses are highly prevalent, particularly in infants from developing countries, both sick and healthy, with frequent co-infections. The rapid rate of viral discovery provides many opportunities for epidemiological and in vivo studies to explore the role of these “new” viruses in both health and disease.

**FEMS-2864**

**Why we need to worry about bacterial infections?**

**Collaborating with the innate immune system to combat multidrug-resistant bacterial pathogens**

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Antibiotic resistance presents an ever increasing challenge to the public health with a dearth of new drugs in the development pipeline. Conventional screening paradigms in antibiotic discovery are based on MIC/MBC testing in conventional bacteriologic media, and similar tests on patient isolates are used to guide physician management. Economic factors have favored development of broad spectrum agents, which exert "collateral damage" on the normal microflora, now increasingly recognized to have adverse health consequences. A single-minded focus on direct antimicrobial activities overlooks the fact that significant infections are really a disease of the host-pathogen interaction. Indeed, before the patient has even seen a doctor, their infection is already being treated by multiple antimicrobials - namely the cellular and molecular components of the innate immune system. We see value in exploring potential novel therapeutic approaches for drug-resistant bacteria that aim to tip the host-pathogen interaction back in favor of the host. This talk will illustrate three such classes of novel therapeutics: (A) Inhibitors of bacterial virulence factors that re-sensitize the pathogen to innate immune killing; (B) Drugs that directly boost the antibacterial killing capacity of host phagocytic cells; (C) Antibiotics that synergies with endogenous antimicrobial peptides to effect bacterial killing. These studies will reveal how standard MIC testing can be misleading, and overlook potent antibiotic activities that are recognized only the context of the normal innate immune system. In this new discovery and treatment framework, drugs used in medicine for other indications, or antibiotics otherwise deemed ineffective, can be "repositioned" for treatment of multi-drug resistant pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* spp. (VRE) or carbapenemase-resistant strains of Gram-negative pathogens including *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*.



**FEMS-2343**

**Why we need to worry about bacterial infections?**

**Meningococemia a disease of the endothelial cells**

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*Neisseria meningitidis*, (also known as the meningococcus), is a common inhabitant of the human nasopharynx, and as such is a normal, saprophytic organism that is transmitted from person to person. These bacteria can therefore spread in a population by direct contact. Paradoxically, in a small proportion of colonized subjects, they disseminate from the nasopharynx into the bloodstream, leading to clinical features, which are rarely observed in bacterial septicemia. First, meningococci usually cross the blood brain barrier, invade the meninges and cause meningitis. Secondly meningococemia is usually associated with a thrombotic syndrome, which causes limited skin purpuric lesions, in addition to the meningeal inflammation in its milder form, or extensive thrombosis and necrotic purpura associated with massive vascular leakage and shock, known as *purpura fulminans*, in its more severe form. The epidemic nature of meningococcal transmission and the high mortality rate, especially in case of *purpura fulminans*, are responsible for the fear of meningococcal infections in communities. *In vivo* observations have clearly shown that *Neisseria meningitidis*, once in the bloodstream, interact closely with the microvessels. This interaction is mediated by the type IV pili. The mechanisms and consequences of this interaction will be analyzed in the light of the clinical consequences.

## FEMS-2402

### Why we need to worry about bacterial infections?

#### Buruli ulcer: from disease to pain control

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Buruli ulcer is an infectious disease transmitted by arthropod vectors harboring *Mycobacterium ulcerans*, a mycobacterium which belongs to the same family of bacteria causing tuberculosis and leprosy. The infection causes painless swelling and severe skin lesions. One key feature of *M. ulcerans* bacterium is its ability to secrete a necrotic toxin, the mycolactone within small lipophilic vesicles, which are critical for the bacterial induced pathogenicity. The biological knowledge as well as the preventive and therapeutic means for this invalidating disease is still very limited.

The extensive skin lesions, despite their severity, are not accompanied by pain. It was previously thought that this remarkable analgesia is ensured by direct nerve cell destruction. We demonstrate that *M. ulcerans*-induced hypoesthesia is instead achieved through a specific neurological pathway triggered by the secreted mycobacterial polyketide mycolactone, leading to potassium-dependent hyperpolarization of neurons. By use of a combination of siRNA and chemical library image based screening, we showed that mycolactone elicits signaling through type 2 angiotensin II receptors (AT2Rs) leading to the activation of cyclooxygenase I and TRAAK potassium channels, which causes neuron desensitization. We further validate the physiological relevance of this mechanism with in vivo studies of pain sensitivity in mice infected with *M. ulcerans*, following the disruption of the identified pathway. Our findings shed new light on molecular mechanisms evolved by natural systems for the induction of very effective analgesia, opening up the prospect of new families of analgesics derived from such systems.

**FEMS-2866**

**Why we need to worry about bacterial infections?**

**The use of antibiotics drive the evolution of resistance by activating competence for DNA uptake: Molecular mechanisms involved in *Streptococcus pneumoniae***

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The rapid spread of antibiotic resistance, combined with a near absence of new antibiotics, are leading to a public health threat. One of the leading bacterial causes of morbidity and mortality worldwide is *Streptococcus pneumoniae* (the pneumococcus). Frighteningly, inappropriate antibiotic treatments can accelerate the occurrence of multidrug resistance by activation of a developmental process called bacterial competence.

In this presentation I will discuss how antibiotics affects pneumococcal physiology and how antibiotics promote competence development. Molecular insights into the mechanisms driving bacterial evolution and resistance will advance the quest for novel treatment strategies.

**FEMS-3164**

**Bacterial predators**

***Bdellovibrio bacteriovorus*- biology of invasive bacterial predation**

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*Bdellovibrio bacteriovorus* is a predatory delta-proteobacterium, with a 3.8Mb genome inside a vibroid cell only 1µm x 0.3µm.

Locomotion by a single flagellum or by a gliding apparatus delivers *Bdellovibrio* to encounter other Gram-negative cells at their outer membrane. There is an attachment (via the non-flagellar pole) and recognition phase and then invasion by *Bdellovibrio* and intraperiplasmic growth, inside the, now-dead, but intact, prey. Growth in the periplasm requires modification of the prey cell wall to prevent premature lysis as the predatory life history strategy of *Bdellovibrio bacteriovorus* is not to share nutrients with bacteria outside the prey. The nutrients available from the prey are finite and dependent on cell volume and even or odd numbers of *Bdellovibrio* progeny are produced. *Bdellovibrio* motility resumes at the end of predator replication to escape the exhausted prey cell by flagellar or gliding motility. The non flagellar pole is specialised for prey-interaction and this involves Type IV pili and associated regulatory proteins, functions for which are becoming understood. This presentation will review the prey-modification and predatory growth processes of *B. bacteriovorus* and touch on their evolution.

**FEMS-0602**

**Bacterial predators**

**When cheese gets the flue: how dairy lactic acid bacteria succumb to bacteriophage attack**

D. van Sinderen<sup>1</sup>

<sup>1</sup>*Microbiology, University College Cork, Cork, Ireland*

Bacteriophages are a constant threat to commercial dairy plants, where their presence may lead to inconsistent, delayed or even disrupted fermentations. Over the last 50 years bacteriophages infecting lactic acid bacteria, and in particular dairy strains of *Lactococcus lactis*, have been isolated and studied to learn about their diversity and biology, as well as to find ways by which their negative impact on their host can be reduced or managed. In recent years, a wealth of information has been generated related to lactococcal host recognition by phages with (non-contractile) tails, representing the vast majority of (dairy-associated) phages. This presentation will present data on recent work in this field, highlighting the role of the saccharide-recognizing base plate located at the tip of the lactococcal phage tail, while also discussing the (diversity of the) phospho-polysaccharide receptor, which is recognized by the base plate.

**FEMS-0600**

**Bacterial predators**

**Structural Biology as a Tool to Study Predatory Bacteria**

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*Bdellovibrio bacteriovorus* is a predatory bacterium that enters other Gram negatives, residing in their periplasm and killing them from within. The goal of my lab is to understand this process on the molecular level, detailing how predation-specific proteins function by use of structural biology. We are currently investigating cell wall metabolism (entry into prey), cyclic-di-GMP signalling (control of predation events), host-independence (conversion to a form that can grow/replicate outside of the prey cell) and a variety of cryptic genes (*Bdellovibrio* has many unique genes, forming the “predatosome”). This presentation will provide examples of our progress in these areas and what we can learn about the toolkit required to live a predatory lifestyle.

**FEMS-2521**

**Bacterial predators**

***Pseudomonas* Predators: A systems' biology perspective of the phage infection process**

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Because of their outspoken ubiquity and versatility, bacterial viruses (phages) constitute an inevitable and integral part of microbial ecology, and are now being recognized as an important selective pressure and/or advantage that continues to drive microbial evolution and behavior. Moreover, the ongoing evolutionary arms race between bacterial hosts and their viral parasites has endowed phages with fine-tuned strategies to interfere with or even reshape microbial physiology. Understanding bacteriophage infection and the impact it has on the bacterial host greatly depends on insights at the systems biology level. Our research focuses on the phage-based identification of new antibacterial targets and indicates a close interplay at the RNA, proteome (interactions & post translational modifications) and metabolome level of *Pseudomonas* phages.

Lytic bacteriophages inject their genome in the bacterial cytoplasm and immediately redirect the host metabolism towards phage genome replication and the production of virions. However, the mechanisms behind the molecular hijack are largely unknown. While bacteriophages infecting *Pseudomonas aeruginosa* have been known and studied for a long time, over 80% of their genes have an unknown function. Recent 'omics' techniques can lead to a drastic increase in the understanding of the struggle between virus and host to dominate the cellular metabolism during all stages of infection. The exploration of four levels of control (transcriptional, posttranscriptional and – translational, protein-protein interactions) and their influence on the bacterial metabolome are the key focus of this presentation. We are currently exploring the potential for biotechnological applications and antimicrobial design strategies of these phage-encoded gene products.

## **FEMS-2809**

### **The Type VI secretion system**

#### **Recent insights into the export and activity of type VI secretion toxins**

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Though they are by definition single-celled organisms, bacteria can exhibit properties more often ascribed to multicellular life. Our group aims to identify, characterize, and eventually, exploit, pathways that bacteria use for intercellular communication and competition. In this presentation, I will discuss our recent progress toward understanding the diversity of mechanisms by which antibacterial toxins delivered by the type VI secretion system act upon recipient cells. I will also describe our discovery that a conserved signaling pathway allows cells of the Gram-negative opportunistic pathogen *Pseudomonas aeruginosa* to mount a coordinated response to antagonism by competing bacteria.



**FEMS-2814**

**The Type VI secretion system**

**The *Pseudomonas aeruginosa* T6SS: a weapon for bacterial warfare**

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Gram-negative bacteria have evolved various strategies to compete in hostile environments. Among them, secretion systems have attracted a lot of attention because of their clinical importance but also because of their complex architecture and regulation. The conserved bacterial type VI secretion system (T6SS) was initially associated with bacteria-host interaction but subsequently found to inject toxins into bacterial targets. These toxins have various activities, the best characterized of which are amidases, phospholipases and DNases.

*Pseudomonas aeruginosa* is an opportunistic pathogen, which has three T6SSs called H1- to H3-T6SS. We used a combination of structural and *in vivo* protein-protein interaction approaches to characterize molecular aspects of the H1-T6SS assembly of *Pseudomonas aeruginosa*. We also characterized several T6SS toxins and studied their mode of delivery and their impact on bacterial competition.

**FEMS-2951**

**The Type VI secretion system**

**Structure, function and dynamics of Type VI secretion system sheath**

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The bacterial Type VI secretion system (T6SS) is a large dynamic organelle that is functionally analogous to contractile tails of bacteriophages. T6SS is used by Gram-negative bacteria to inhibit adjacent cells via translocation of toxic effector proteins and thus play an important role in bacterial ecology. Time-lapse fluorescence light microscopy revealed that T6SS sheath, which powers the secretion, cycles between assembly, quick contraction, disassembly and re-assembly. Single cell analysis of subcellular localization of T6SS assembly revealed that T6SS organelle encoded by *Pseudomonas aeruginosa* H1-T6SS cluster is assembled and aimed to specifically retaliate against attack by other bacteria. I will present latest update on the structure, function and dynamics of T6SS as well on mechanisms of effector delivery. I will focus on the structure of the T6SS sheath and the implications for T6SS dynamics and assembly.

## **FEMS-0625**

### **Bacterial spores**

#### **Germination of spores of *Bacillus subtilis*: nothing but surprises**

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#### **Background**

Previous work has identified proteins essential for *Bacillus subtilis* spore germination as the: i) germinant receptors (GRs) that recognize nutrient germinants; ii) GerD protein essential for rapid GR-dependent germination; iii) SpoVA proteins that likely comprise a channel for dipicolinic acid (DPA) release early in germination; and iv) lytic enzymes that degrade the peptidoglycan cortex later in germination. Previous work has determined these proteins' locations in spores, some of their structures and some knowledge of how their function is regulated.

#### **Objectives**

Identify and understand the earliest spore germination events.

#### **Methods**

To help answer these latter questions we have examined germination of multiple individual *B. subtilis* spores under various conditions.

#### **Conclusions**

Surprising recent findings from this work include the following. 1) At approximately when spores become committed to germinate, spores' inner membrane permeability decreases and DPA begins to leak out, with this leakage more evident at higher temperatures. Following leakage of 10-20% of spores' DPA, the remainder is released in 1-2 min. The change leading to slow DPA leakage is unknown, but could be changes in spores' inner membrane, the DPA channel, or both. 2) Spores have memory of exposure to nutrient germinants, such that a 1<sup>st</sup> germinant pulse results in more germination following a 2<sup>nd</sup> germinant pulse. This memory is established before commitment, as memory is lost upon extended incubation, especially at elevated temperatures. Memory is formed by stimulation of any GR and can be accessed by stimulation of any GR. However, how spore memory is generated, stored and accessed are not known.

**FEMS-2319**  
**Bacterial spores**

**The polar septum - the way how to set up asymmetry during sporulation in Bacilli and Clostridia**

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A general problem in developmental biology concerns the process by which cells of one type divide to give dissimilar daughter cells. Even though these daughter cells may be genetically identical, they can differ morphologically and physiologically and have different fates. As one of the simplest differentiation processes, *Bacillus subtilis* sporulation represents an excellent model system for studying cell differentiation. Several decades of study of this process have provided insight into cell cycle regulation and development. The first clear morphological step in sporulation is formation of division septum at the polar site, which is fundamental to the development of highly resistant endospores formed by bacterial species as Bacilli and Clostridia. The earliest visible event in this asymmetric cell division is the formation of a Z-ring by FtsZ, a tubulin like protein, and E-ring by SpoIIIE at the future sporulation septum site.

There are many important advances in our understanding of asymmetric gene expression during spore formation with an emphasis on developmental stages that lead to asymmetric septum formation and especially to activation of the first compartment specific sigma factor - sigF. It is clear that SpoIIIE is a crucial constituent of the sporulation septum formation and an important determinant of the proper activation of sigF. However, we understand these processes only partially and there are few fascinating unanswered questions as: how SpoIIIE localises to the polar septum; how SpoIIIE causes FtsZ to relocate from mid-cell to the polar site; how SpoIIIE's phosphatase activity is controlled so that sigF activation is delayed until the septum is completed; what role SpoIIIE plays in septal thinning or what is the role of other proteins and Min system in this stage of sporulation.

## **FEMS-0590**

### **Bacterial spores**

#### **The role of spores in *C. difficile* infection**

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*Clostridium difficile* infection (CDI) is an important nosocomial infection in the developed world and one for which no vaccine currently exists. In the USA alone about 28,000 people die each year and of these most occur from nosocomial infection. It is universally accepted that extensive antibiotic usage promotes germination of *C. difficile* spores in the GI-tract followed by secretion of toxins (A and B) from the live bacterium that then produce symptoms of disease (gastroenteritis and diarrhea). *C. difficile* is a spore-forming bacterium and both humans and animals can be asymptomatic carriers. It is also accepted that *C. difficile* can be acquired from the environment and there is evidence of transfer of animal strains (e.g., the 078 ribotype) to humans.

While the vegetative bacterium produces symptoms of disease there is compelling evidence that other virulence factors exist and that the spore plays an important role in infection. For example, oral administration of non-toxin producing spores confer protection to CDI in humans implying that other correlates of infection exist.

This talk will focus on two aspects of CDI. First, the characterization of proteins found on the surface of *C. difficile* spores and second, strategies we are using to vaccinate against CDI using mucosal delivery of *C. difficile* antigens and that are now in clinical trial.

Analysis of spore coat proteins has revealed a number of proteins that carry enzymatic properties of which one can be linked to inflammation. A second, BclA1, has been shown to play a distinct role in the initial stages of infection potentially by recognition of a specific receptor molecule in the gut mucosa.

**FEMS-2846**

**Bacterial spores**

**Structural analysis of proteins involved in peptidoglycan hydrolysis during spore germination**

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A key event in the germination of spores of *Bacillus* and *Clostridium* species concerns depolymerisation of the thick layer of cortical peptidoglycan that surrounds the spore core. Cortical dissolution is achieved via the activities of a group of peptidoglycan lysins referred to as cortex lytic enzymes (CLEs). CLEs can be further sub-divided into those that cleave intact peptidoglycan, and those that function as cortical-fragment lytic enzymes (CFLEs). The molecular mechanisms that underpin the transition of inactive CLEs and CFLEs in the dormant spore, to catalytically active enzymes during spore germination, are poorly understood. In the current work we have used X-ray crystallography to examine CFLEs from both *Bacillus* (SleL) and *Clostridium* (SleM) spores in an attempt to gain structural insights to the regulation of CFLE activity. To date, high-resolution structural information has permitted relatively facile identification of the active sites and catalytic residues in the respective enzymes, and has revealed why SleL from *B. cereus* spores is significantly more catalytically efficient than the orthologous *B. megaterium* enzyme. Determination of the *C. perfringens* SleM crystal structure has additionally revealed unusual structural features that are probably involved in substrate or protein interactions. Finally, comparison of SleL and SleM crystal structures with the *Bacillus* CLE, SleB, has permitted the generation of hypotheses regarding the structural basis for CFLE substrate selectivity, which will be discussed during the presentation.

## **FEMS-1217**

### **Insect-microbes interactions**

#### **Small RNA-based antiviral immunity in insects: defense and counter-defense**

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The RNA interference (RNAi) pathway senses virus infection to induce an antiviral response in insects. The ribonuclease Dicer-2 detects viral double-stranded RNA and generates viral small interfering RNAs (vsiRNAs), which are then used to restrict viral replication. Recent small RNA profiling studies demonstrated that both RNA and DNA viruses are targets of the RNAi response and identified the viral RNA substrates that trigger vsiRNA biogenesis. The importance of the antiviral RNAi pathway is underscored by the observation that viruses have evolved sophisticated mechanisms to counteract this immune response. More recently, it was proposed that another small RNA silencing mechanism, the piRNA pathway, also processes viral RNAs in *Drosophila* and mosquitoes. I will present recent insights into antiviral RNAi and discuss the dynamic interplay between host antiviral RNAi responses and virus counter-defense strategies.

**FEMS-2554**

**Insect-microbes interactions**

**The *Drosophila*-*Spiroplasma* interaction as a model to dissect the molecular mechanisms underlying insect endosymbiosis**

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Virtually every species of insect harbors facultative bacterial endosymbionts that are transmitted from females to their offspring, often in the egg cytoplasm. These symbionts play crucial roles in the biology of their hosts. Many manipulate host reproduction in order to spread within host populations. Others increase the fitness of their hosts under certain conditions. For example, increasing tolerance to heat or protecting their hosts against natural enemies. Over the past decade, our understanding of insect endosymbionts has shifted from seeing them as fascinating oddities to being ubiquitous and central to the biology of their hosts, including many of high economic and medical importance. However, in spite of growing interest in endosymbionts, very little is known about the molecular mechanisms underlying most endosymbiont-insect interactions. For instance, the basis of the main phenotypes caused by endosymbionts, including diverse reproductive manipulations or symbiont-protective immunity, remains largely enigmatic. To fill this gap, we are dissecting the interaction between *Drosophila* and its native endosymbiont *Spiroplasma poulsonii*. This project will use a broad range of approaches ranging from molecular genetic to genomics to dissect the molecular mechanisms underlying key features of the symbiosis, including vertical transmission, male killing, regulation of symbiont growth, and symbiont-mediated protection against parasitic wasps. We believe that the fundamental knowledge generated on the *Drosophila*-*Spiroplasma* interaction will serve as a paradigm for other endosymbiont-insect interactions (ex. *Wolbachia*) that are less amenable to genetic studies.



**FEMS-0628**

**Insect-microbes interactions**

**How gut microbes interact with animals: insights from *Drosophila***

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**Background:** Animal surfaces and cavities provide multiple habitats for those microorganisms that can tolerate the conditions and exploit the resources provided by their animal hosts. Many of these microorganisms display adaptations for the host habitat, some of which are co-evolved with metabolic or immunity-related traits of the host. Teasing apart these interactions represents a major challenge, especially in humans and other mammals with very complex physiological systems and diverse microbial communities.

**Objectives:** To use the relationship between *Drosophila* and its gut microbiota as a model system to investigate the fundamentals of interactions between animals and their gut microbiota.

**Methods:** We have quantified the impact on the association of systematically varying the diet composition, microbiota composition and host genotype, using multiple methods including analysis of (meta)-genome-wide associations

**Conclusions:** Key traits that are influenced by host and microbial genetic factors are the abundance of the microorganisms in the gut and the energy status of the host, including metabolic rate and fat content. Our first datasets also point to the importance of among-microbe interactions in shaping the total function of the microbial community. Because the microbial community in *Drosophila* is relatively simple, it is feasible to investigate these interactions systematically.

**FEMS-1378**

**Insect-microbes interactions**

**Digesting the diversity: Ecology and evolution of the termite gut microbiota**

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Termites degrade lignocellulose with the help of their intestinal symbionts. The general role of the gut microbiota in the digestive process is slowly emerging, but the specific functions of individual populations and their evolutionary origin are still in the dark. Deep sequencing of the hindgut community revealed strong differences among the major host groups, and dramatic changes in the abundance of particular taxa coincide with major events in termite evolution. The acquisition of cellulolytic protists by an ancestral cockroach provided new habitats for bacterial symbionts, which colonize the surface and cytoplasm of the gut flagellates in all evolutionary lower termites. After the loss of flagellates in higher termites, the wood particles became available for bacterial colonization, providing new niches for fiber-digesting populations. While some lineages in the core microbiota may be cospeciating with termites since the early Cretaceous, others seem to be specifically selected by the microenvironmental conditions in insect guts. Experiments addressing the role of the host in the selection of its gut microbiota are hampered by the elaborate social system of termites and their obligate dependence on intestinal symbionts. However, the controlled inoculation of germ-free cockroaches with normal and foreign microbiota confirmed that host factors are important drivers shaping the gut microbial community.

**FEMS-3160**

**Bacterial cell biology**

**Mechanisms and evolution of bacterial morphology**

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The diversity of shapes of organisms is one of the most fascinating aspects in the field of biology. While bacteria display a myriad of morphologies, the mechanisms that control morphogenesis and the evolution of bacterial morphology are not understood. I will describe the mechanisms that control morphological diversity in species related to *Caulobacter crescentus* that synthesize appendage-like extensions of the cell envelope at distinct sub-cellular positions. I will show that stepwise evolution of a specific domain of a developmental regulator led to the gain of a new function and localization of this protein, which drove the sequential transition in morphology. Our results indicate that evolution of protein function, co-option, and modularity are key elements in the evolution of bacterial morphology. In addition, I will show how evolutionary consideration of the mechanism of growth in the alphaproteobacteria led to the surprising discovery that polar growth, rather than the previously assumed binary fission, is the predominant mode of growth in a large group of the alphaproteobacteria that includes the plant pathogen *Agrobacterium tumefaciens* and the human pathogen *Brucella abortus*. Finally, I will describe new methods of peptidoglycan labeling that allow the detection of sites of peptidoglycan synthesis in live cells and in real time, and their use to study the mechanisms of peptidoglycan synthesis and to show for the first time that pathogenic Chlamydia have peptidoglycan, ending 50 years of speculation and debate concerning the chlamydial anomaly.

**FEMS-2843**

**Bacterial cell biology**

**Bacterial chromosome segregation at the single-molecule resolution**

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**Bacterial chromosome segregation at the single-molecule resolution**

How does a bacterial cell without a nucleus, without mitosis, and without extensive sister cohesion, organize, replicate, repair and segregate its chromosomes? We use live-cell super-resolution PALM and 3-D SIM imaging, alongside classical biochemistry and molecular genetics, and *in vitro* single-molecule biophysical techniques to address these questions. The presentation will focus on the sequential and coordinated action of Topoisomerase IV and the SMC complex, MukBEF, in *E. coli* chromosome segregation.

*References*

*Science* (2012) **338**, 528-531

*PNAS* (2013) **110**, 8063-8068

*Nature* (2014) **506**, 249-253

**FEMS-0626**

**Bacterial cell biology**

**Regulation of cell division in bacteria**

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Background: Accurate positioning of the division site is essential for generating appropriately-sized daughter cells with the correct chromosome number. In bacteria, cell division initiates with assembly of the tubulin homologue FtsZ into a ring-like structure, the Z-ring. Thus, the position of Z-ring formation dictates the cell division site. Accordingly, all known systems that regulate positioning of the division site control Z-ring positioning.

Objective: We analyzed how the rod-shaped *Myxococcus xanthus* cells regulate positioning of the Z-ring at midcell.

Methods: Genetics, microscopy and *in vitro* analyses of proteins.

Conclusions: We report that PomX, PomY and the ParA-like ATPase PomZ are important for formation and positioning of the Z-ring. PomXYZ colocalize in a dynamically localized cluster and depend on each other for localization in this cluster. Early during the cell cycle this cluster localizes on the nucleoid away from midcell. Subsequently, the cluster relocates by directed motion to midcell and localizes here before and in the absence of FtsZ. PomZ stimulates the movement of the PomXYZ cluster to midcell in an ATP hydrolysis-dependent manner. *In vitro* PomX and PomY interact and stimulate PomZ ATPase activity. Moreover, PomY and PomZ separately inhibit FtsZ filament formation. We conclude that *M. xanthus* uses a novel system to precisely mark midcell for Z-ring formation and cell division. We propose that Z-ring formation is inhibited throughout cells by PomY and PomZ. At midcell, PomXYZ establish a zone that is permissive to Z-ring formation either by inhibition of PomYZ function and/or by direct stimulation of Z-ring formation.

## FEMS-0802

### Bacterial cell biology

#### The PALM nanostructure of FtsZ along the cell cycle of a pathogenic coccus

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Ovococci form a morphological group that includes several human pathogens (streptococci and enterococci). Their shape results from two modes of cell wall insertion, one allowing division, and another one allowing limited elongation. Both cell wall synthesis modes rely on a single cytoskeletal protein, FtsZ, since MreB is absent in ovococcal bacteria. Despite the central role of FtsZ in ovococci, a detailed view of the *in vivo* nanostructure of ovococcal Z-rings has been lacking thus far, limiting our understanding of their assembly and architecture.

We have developed the use of PhotoActivated Localization Microscopy in the ovococcus human pathogen *Streptococcus pneumoniae* by engineering spDendra2, a photoconvertible fluorescent protein optimized for this bacterium. Labeling of endogenously expressed FtsZ with spDendra2 revealed the remodeling of the Z-ring morphology along the division cycle at the nanoscale. Changes in the ring axial thickness and in the clustering propensity of FtsZ correlated with the advancement of the cell cycle. We observed double-ring sub-structures, suggestive of short-lived intermediates that may form upon initiation of septal cell wall synthesis. The nanostructural details provided in this work have been integrated in a model describing the morphology of the ovococcal Z-ring along the cell cycle.

**FEMS-3157**  
**Closing Session**

**The Lwoff Award Presentation: The microbial methane cycle**

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Globally, each year about 270 Gt CO<sub>2</sub> are net fixed via photosynthesis into biomass, mainly lignocelluloses. Of this biomass about 2% end up in anoxic environments low in sulfate, Fe<sup>3+</sup>, Mn<sup>4+</sup>, nitrate and/or nitrite, where biomass is fermented via syntrophic associations of anaerobic protozoa, fungi, bacteria and archaea to methane and CO<sub>2</sub>. The methane thus generated either accumulates in the sediments forming methane hydrates or diffuses into zones where the electron acceptors sulfate, Fe<sup>3+</sup>, Mn<sup>2+</sup>, nitrate, nitrite and/or O<sub>2</sub> are available and where methane is oxidized by anaerobic archaea and aerobic bacteria to CO<sub>2</sub>. Part of the methane also escapes into the atmosphere, where it is photochemically converted to CO<sub>2</sub>, and where methane is a potent greenhouse gas, which is of concern since the methane concentration in the atmosphere has doubled within the last 100 years. Many of the microbes involved in the methane cycle display a unique biochemistry, which my group helped to unravel within the last 50 years. In my lecture I will focus on the function and structure of the nickel enzyme methyl-coenzyme M reductase, which catalyzes in methanogenic archaea the methane forming step and in methanotrophic archaea the methane oxidizing step. The enzyme contains uniquely the nickel porphinoid F<sub>430</sub> as prosthetic group and, highly conserved, six postranslationally modified amino acids within the active site, amongst them a thioglycine, two C-methylated amino acids and an only recently discovered didehydroaspartate. The function of the posttranslational modifications has remained enigmatic and therefore also the catalytic mechanism of MCR.

Thauer, R. (2015) My lifelong passion for biochemistry and anaerobic microorganisms. *Annu. Rev. Microbiol.* 69: in press. doi: 10.1146/annurev-micro-091014-104344

## **FEMS-1907**

### **Poster Discussion: Discovery of Novel Antibiotics**

#### **THE HUMAN GUT AS A RESERVOIR FOR ANTIBIOTIC RESISTANCE GENES**

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#### **Background**

The human gut harbors a densely populated microbial ecosystem, termed the gut microbiota. The gut microbiota can serve as a reservoir for antibiotic resistance genes ('the resistome') and drug-resistant opportunistic pathogens.

#### **Objectives**

In this study, we determined the dynamics of the microbiota and the resistome in thirteen patients that were hospitalized in Intensive Care Units. Because gut-associated bacteria and their resistance genes may be shed into the environment through sewage, we also assayed the levels of antibiotic resistance genes in hospital sewage and in subsequent stages of wastewater treatment.

#### **Methods**

16S rRNA profiling, functional metagenomics, metagenomic sequencing and nanolitre-scale quantitative PCRs were used in this study.

#### **Conclusions**

The gut microbiota of hospitalized patients contained genes that are predicted to confer resistance to fourteen classes of antimicrobials. Levels of antibiotic resistance genes increased during ICU hospitalization and were associated with changing patterns of the composition of the microbiota. Sequencing of vector inserts in drug-resistant fosmid clones indicated that resistance genes were mainly carried by anaerobic gut commensals (*Clostridium* and *Bacteroides*). Resistance genes were associated with mobile genetic elements, which could facilitate horizontal gene transfer of resistance genes from commensal bacteria to opportunistic pathogens. Antibiotic resistance genes were abundant in hospital sewage but the influence of hospital sewage on the abundance of resistance genes in the wastewater treatment



plant was limited. Levels of resistance genes and human-associated bacteria further decreased during wastewater treatment, limiting the dispersal of antibiotic resistance genes from hospitals into the environment.

**FEMS-2761**

**Poster Discussion: Discovery of Novel Antibiotics**

**SYNTHETIC BIOLOGY FOR THE DESIGN OF ANTIMICROBIAL PEPTIDES**

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Regensburg, Germany

**Background**

The number of multi drug resistant pathogens is constantly growing and novel antibiotic substances are desperately needed in order to at least maintain the status quo. Ribosomally synthesized antimicrobial peptides are not yet exploited for human applications despite an indisputable potential. Among those, lantibiotics, a class of posttranslationally modified and thioether-ring bearing antimicrobial peptides, show desirable properties as high but specific antimicrobial activity and excellent stability.

**Objectives**

We follow a synthetic biology approach for the generation of novel lantibiotics employing the blueprint of natural lantibiotics. Based on their structural and functional features, natural lantibiotics are dissected into modular subunits. These subunits are then shuffled to generate thousands of novel, putative active, chimeric lantibiotics. The lantibiotic genes are subsequently produced by combinatorial DNA *de novo* synthesis. These libraries will then be screened for molecules with high antimicrobial activity.

**Methods**

To enable for high screening rates we developed a platform based on nL-sized reaction vessels (nL-reactors) that are used for peptide production and activity-screening in a single step and at rates of 10<sup>5</sup> variants per day. Library cells are grown to microcolonies within nL-reactors along with a sensor strain. Cells secreting an active antimicrobial peptide will deactivate the sensor cells. Clearance of an nL-reactor from the sensor thus indicates the presence of a strain secreting a highly active peptide.

**Conclusions**

10<sup>4</sup> different chimeric variants derived from the module-shuffling of ten natural lantibiotics were analyzed. Several interesting antimicrobials have been isolated, demonstrating for the first time the generation of new-to-nature lantibiotics by module-shuffling.

**FEMS-2987**

**Poster Discussion: Discovery of Novel Antibiotics**

**DOUBLE LEADING: LANTIBIOTIC - MICROCIN HYBRIDS**

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**Background**

Lantibiotics and microcins form different groups of posttranslationally modified bacterial peptides mainly produced by Gram-positive and Gram-negative bacteria, respectively. Most of these peptides exhibit a potent antimicrobial activity, more active even than some antibiotics. Maturation of a peptide begins with a propeptide which is guided throughout specific modification events due to leader peptide (an amino acid sequence recognized by particular modification biomodule). The leader peptide is proteolitically removed in the last steps of peptide modification making the modified peptide active. Posttranslational modifications endorse lantibiotics and microcins with high target-specific activities and stability against proteolysis.

**Objectives**

To design plug-and-play biomodules for production of novel peptides containing both, lantibiotic- and microcin-specific posttranslational modifications.

**Methods**

We used a synthetic biology approach to design biomodules. We utilized peptide modification systems cloned under different controlled promoters to assure gradual and temporal activity of designed biomodules. Codon optimized synthetic genes of designed model peptides to be modified were employed together with modification machineries.

**Conclusions**

This is a unique case where posttranslational modifications from two different peptide classes are fused together into a single peptide chain *in vivo*. Here, we also show the possibilities of employing chimeric leader peptides for substrate recognition and procession by particular posttranslational modification machineries.

**FEMS-2414**

**Poster Discussion: Discovery of Novel Antibiotics**

**MECHANISMS OF ANTIBACTERIAL ACTION OF CATIONIC PEPTIDE  
WARNERIN**

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**Background**

Rapid development of antibiotic resistance of bacteria necessitates the search for new natural compounds inhibiting the growth and functioning of pathogenic microflora.

**Objectives**

To study the antibacterial mechanisms of warnerin, a new peptide from lantibiotic family that demonstrate the sequence of events causing the death of attacked bacterial cells.

**Methods**

Outcomes of warnerin attack of *Staphylococcus epidermidis* 33 GISK (Moscow) were analyzed using the combination of different methods such as polarography (respiration), spectrography (intracellular potassium loss), fluorescence assay of membrane potential dynamics and hydroxyl radicals' formation, electron and atomic-force microscopy of cell structure damages

**Conclusions**

Peptide introduction into the bacterial incubation medium results in the increase in their oxygen consumption followed by rapid cellular release of potassium ions and sharp decline of intracellular ATP concentration. Apparently, the disturbance of ATP-linked electron transport along the respiratory chain favors their accumulation on iron-sulfur centres. This results in increase of free Fe<sup>2+</sup> ions and as a consequence, to the elevation of hydroxyl radical concentrations. Peptide inhibition of ATP formation is accompanied by marked proton accumulation on the outer side of bacterial membrane, particularly in its adjacent layers of peptidoglycane. This facilitates the autolytic hydrolase extrusion from the binding sites and to uncontrolled total bacteriolysis that is revealed by microscopic data. Results of investigation evidence for the pluripotent antibacterial action of warnerin that point to expressed prospects for its practical use.

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**FEMS-1787**

**Poster Discussion: Discovery of Novel Antibiotics**

**ISOLATION AND CHARACTERIZATION OF NOVEL BACTERIOPHAGES WITH  
LYTIC ACTIVITY AGAINST MULTIDRUG RESISTANT PATHOGENS**

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**Background**

With the emerging threat of infections caused by multidrug-resistant bacteria and scarce prospects of newly introduced antibiotics in the future, bacteriophages (phages) are currently being reconsidered as alternative therapeutics. However, the full breath of phage diversity suitable for treatment of bacterial infections is still largely unexplored.

**Objectives**

This study aims at providing an overview of the general occurrence of bacteriophages (with therapeutic potential) in natural environments and at devising strategies for their rapid characterization and classification at molecular level.

**Methods**

Novel phages were isolated from various aquatic systems (e.g. from general and hospital wastewater, activated sludge samples from sewage plants, streams, rivers, ponds and lakes). Environmental samples were pre-incubated with clinical isolates of multidrug-resistant bacteria. Enriched bacteriophages were subsequently obtained with the double agar layer plaque assay. "PhiSigns"

(<http://www.phantome.org/phisigns/>) was used for phage identification based on the detection of signature genes. Genomic comparison of phages was performed based on digitized fluorescent restriction-fragment-length-polymorphism-analysis (fRFLP).

**Conclusions**

For roughly 50% of tested bacterial strains lytic bacteriophages were found against multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterobacter* sp. with waste water samples harbouring the highest phage diversity. By contrast no bacteriophages were isolated against multidrug-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*. PhiSigns combined with fRFLP proofed to be a useful tool for rapid phage identification, which is the base for their proper selection for ultimate whole genome sequencing. *S. aureus* and *A. baumannii* phages require additional isolation steps such as FeCl<sub>3</sub>-precipitation and/or use of

subinhibitory antibiotic-concentrations for enhancing plaque visibility on agar plates.

FEMS-2648

Poster Discussion: Discovery of Novel Antibiotics

**THERAPEUTIC MONOCLONAL ANTIBODIES AGAINST THE GLOBALLY SPREAD MULTI-DRUG RESISTANT ESCHERICHIA COLI CLONE ST131-O25B:H4**

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**Background**

The globally spread multi drug resistant *E. coli* clone ST131-O25b:H4 is responsible for a significant proportion of multi-drug resistant extraintestinal infections. By definition, isolates of this clone express the O25b antigen, whose unique structure has been resolved recently.

**Objectives**

We aimed to develop humanized monoclonal mAbs against the O25b antigen that may provide an alternative adjunct or stand-alone therapeutic option against this *E. coli* lineage.

**Methods**

Murine mAbs against the unique O25b antigen were generated by standard hybridoma technique. Following a primary screening, selected antibodies were subjected to humanization. Specificity and binding characteristics were assessed by immunoblots and ForteBio using purified antigens. Surface staining of ST131-O25b isolates irrespective of the capsular type expressed was shown by flow cytometry. Upon surface binding, the humanized antibodies induced complement mediated bactericidal activity *in vitro*. Passive immunization with the humanized mAbs elicited high level of protection at low mAb doses in a murine model of bacteremia. All whole cell assays were performed with bacteria grown in standard culture medium as well as in depleted pooled human serum in order to mimic *in vivo*-like conditions.

**Conclusions**

Humanized mAbs against *E. coli* O25b antigen were developed that elicited bactericidal activity *in vitro* and showed protection *in vivo*. Prophylactic passive immunization of colonized individuals or adjunct therapy of infected patients by mAbs may replace/substitute antibiotic therapy against this drug resistant clone. Relevant cases could be identified by the co-developed companion diagnostic tool.



**FEMS-0726**

**Poster Discussion: Virology**

**LACTOCOCCUS GARVIEAE'S VIRUSES: AN INSIGHT INTO TEMPERATE AND VIRULENT BACTERIOPHAGES**

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**Background**

*Lactococcus garvieae*, one of the most important pathogens in the aquaculture sector, has been recently recognized as an opportunistic human pathogen. However, little is known about the factors controlling of its growth.

**Objectives**

The aim of this project is to isolate and characterize temperate and virulent phages of *L. garvieae*.

**Methods**

Forty-five *L. garvieae* strains isolated from various ecological niches were tested for the presence of inducible prophages. Virulent phages were also isolated from different environments. Morphological and genomic characteristics of the new phages were studied through transmission electronic microscope (TEM), DNA restriction profile, protein profile, host range, and in few cases, genomic sequences.

**Conclusions**

Twenty temperate phages belonging to the *Siphoviridae* family and five new virulent phages able to infect *L. garvieae* strains have been isolated and partially characterized. Temperate phages, integrated into the genome of the host cell, promoted genome plasticity and appeared to be involved in gene mobilization. Virulent and strain-specific phages may be useful as alternatives to antibiotics to treat *L. garvieae* infections.

**FEMS-1230**

**Poster Discussion: Virology**

**ALGAL VIRUS PRODUCTION IS NEGATIVELY AFFECTED BY PHOSPHORUS AND NITROGEN LIMITATION, BUT THIS MAY BE COUNTERACTED BY NUTRIENT ASSIMILATION DURING INFECTION**

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**Background**

Viruses are a main cause of phytoplankton mortality, thereby controlling their community composition and driving biogeochemical cycling in the marine environment. Phytoplankton growth and production is often limited by nitrogen (N) and/ or phosphorus (P), which can affect the virus growth characteristics upon infection. The influence of nutrient limitation on phytoplankton – virus interactions are, however, still poorly studied. As climate change will lead to an expansion of stratified and consequently nutrient limited oceanic regions, it is important to understand how phytoplankton mortality rates in the future marine environment will be affected by these changes.

**Objectives**

Our goal was to study the effects of N- and P-limitation on virus host interaction and the potential relieve of these effects by the uptake of nutrients during viral infection.

**Methods**

Therefore, cultures of the the phytoplankton species *Micromonas pusilla* and *Phaeocystis globosa* were grown semicontinuously under limitation of either P, N, or NP until the moment of infection. Upon infection the abundances of host cells and viruses were monitored to obtain one-step growth curves.

**Conclusions**

Nutrient limitation led to elongated viral latent periods (up to 3-fold) and strongly reduced (up to 8-fold) burst sizes, whereby the outcome was dependent on both the species and the limiting nutrient involved. Furthermore, the negative effects of P-limitation on virus production in the picophytoplankter *M. pusilla* could be overcome by spiking the infected and P-limited cultures with small pulses of inorganic and organic P, simulating bacterial remineralization and supply by lysis of neighboring cells, respectively.

**FEMS-2787**

**Poster Discussion: Virology**

## **REMARKABLE DIVERSITY AMONG NOVEL DENSOVIRUSES FROM CRICKETS**

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### **Background**

Densoviruses are members of the Parvoviridae family infecting invertebrates. Since 2009, a cricket densovirus, AdDV, caused a severe epidemic in the \$ 600-million cricket industry in North-America.

### **Objectives**

During the last 18 months, we also received samples of diseased crickets from North America, Europe and Japan that were negative for AdDV. Electron microscopy demonstrated that these samples contained densovirus-like particles. Objectives of this study included characterization of these virus(es), cloning, sequencing, and X-ray crystallography.

### **Methods**

A SISPA method was used to detect the pathogens (J Virol 88:12152). Sequencing of the amplicons identified those with identities to known viruses allowing by primer extension to obtain the complete genomes and cloning in suitable vectors to obtain infectious clones. Sequencing revealed that some were novel circoviruses and a novel ambisense densovirus (Genome Announc. 1(2):e00079-13, 1(3):e00328 and 131(6):e00914-13). However, the greatest surprise was a densovirus with a segmented genome (AdSDV). NS- and VP- coding sequences are in separate segments of about 3.3 kb. Both NS- and VP- segments are flanked by identical hairpin terminal sequences but lack ITRs. The two ORFs for VP arose through recombination between a mosquito brevidensovirus and AdDV. The downstream ORF with AdDV-VP1up-like phospholipase A2 could be spliced to the C-terminus of the major capsid protein. Currently, the capsid structure (X-ray crystallography) and transcription strategy of AdSDNV.

### **Conclusions**

A brevidensovirus that normally infects only mosquitoes has adapted itself through recombination with AdDV to crickets. These findings necessitate a revision of the definition and taxonomy of parvoviruses.

**FEMS-1562**

**Poster Discussion: Virology**

**VACCINE-TYPE ATTENUATING MUTATIONS IN HIGHLY PASSAGED STRAIN OF VARICELLA ZOSTER VIRUS**

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Background: Varicella-zoster virus (VZV) is causative agent for chickenpox and zoster. Live attenuated vaccines have been developed based on Japanese Oka strain and Korean MAV/06 strain. Comparison of the complete sequences between vaccine and wild-type strains suggested at least 24 mutations important for attenuation in vaccine strains. Certain strains with high in vitro passage history such as the strains Ellen and 32p72 contained some of the vaccine-type mutations. Objective: In this study, we attempted to understand vaccine-type attenuating mutations in highly passaged VZV strains.

Methods: Genome sequences of clinical strains with different passages were determined by next-generation sequencing and compared with those of other VZV strains.

Conclusion: Vaccine-type mutations were found at position 106262 (T→C) in the Korean clinical strain YC01 passage 32 and at positions 560 (T→C), 105169 (A→G), 106262 (T→C) and 107252 (T→C) in YC01 passage 61. Similar mutations were also found in high-passaged another clinical strain YC02 at positions 560, 106262 and 107252. Same mutations at 106262 and 107252 were found in the strains Ellen and 32p72. Thus, in the course of in vitro passaging of VZV, mutations at 106262 and 107252 seemed to occur first, and followed by mutation at 560. Direct PCR sequencing of these positions in YC01 and YC02 strains at various passages identified the passage numbers when the attenuating mutations occurred. Further studies of in vitro passaging under attenuating conditions such as low temperature or guinea pig cells will help to understand the mechanism of attenuating mutations in VZV.

**FEMS-2207**

**Poster Discussion: Virology**

**TIME SINCE ONSET OF DISEASE AND INDIVIDUAL CLINICAL MARKERS  
ASSOCIATE WITH TRANSCRIPTIONAL CHANGES IN UNCOMPLICATED  
DENGUE**

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**Background**

Dengue virus (DENV) infection causes viral haemorrhagic fever that is characterized by extensive activation of the immune system.

**Objectives**

The aim of this study is to investigate the kinetics of the transcriptome signature changes during the course of disease and the association of genes in these signatures with clinical parameters.

**Methods**

Sequential whole blood samples from DENV infected patients in Jakarta were profiled using affymetrix microarrays, which were analysed using principal component analysis, limma, gene set analysis, and weighted gene co-expression network analysis.

**Conclusions**

Time since onset of disease associates with the shift in transcriptome signatures from immunity and inflammation to cell cycle and repair mechanisms in patients with non-severe dengue. The strong association of time with blood transcriptome changes hampers both the discovery as well as the potential application of biomarkers in dengue. Clinical diagnosis (according to the WHO classification) does not associate with differential gene expression. However, network analysis did show that that key clinical markers, including platelet count, fibrinogen, albumin, IV fluid distributed per day and liver enzymes SGOT and SGPT, strongly correlate with gene modules that are enriched for genes involved in the immune response. The expression level of these gene modules may support earlier detection of disease progression as well as clinical management of dengue.

Ref: CAM van de Weg\*, HJ van den Ham\*, MA Bijl, F Anfasa, F Zaaraoui-Boutahar, BE Dewi, L Nainggolan, WFJ van IJcken, ADME Osterhaus, BEE Martina, ECM van Gorp, AC Andeweg. PLoS Neg Trop Dis 2015

**FEMS-1419**

**Poster Discussion: Virology**

## **CHARACTERIZING THE VIROME OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA**

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### **Background**

Entomopathogenic fungi are of scientific interest because they enable analysis of virus-host interactions and can be used as biocontrol agents of insect pests. The deuteromycetous fungus *Beauveria bassiana* has a widespread geographical distribution and a wide host range. Mycoviruses have been described mostly in phytopathogenic fungi and are occasionally associated with hypovirulence, while in entomopathogenic fungi presence of mycoviruses has been reported only rarely.

### **Objectives**

The aim of the present study is to detect and analyse the viruses present in a collection of *B. bassiana* isolates sourced from worldwide locations.

### **Methods**

Population studies, RT-PCR, cloning and sequencing, hybridization experiments, time-course studies and transmission electron microscopy (TEM) were used to characterize the virome of *B. bassiana*.

### **Conclusions**

A population study revealed that 17/75 (22.7%) *B. bassiana* isolates harbor dsRNA elements. Two members of the *Partitiviridae* family, *B. bassiana* partitivirus-1 and -2 (BbPV-1 and BbPV-2), have been sequenced, while TEM revealed the presence of virus-like particles. Hybridization experiments revealed that BbPV-1 and BbPV-2 are present in seven *B. bassiana* isolates derived from different hosts and geographical origins. Additionally, four members of the *Totiviridae* family, originating from the Iberian Peninsula and the Canary Islands, have been partially sequenced. Furthermore, two previously uncharacterized viruses, *B. bassiana* polymycovirus-1 (BbPmV-1) and *B. bassiana* non-segmented virus-1 (BbNV-1), have been detected and fully sequenced and the prevalence of further PmV-like viruses in *B. bassiana* is currently under investigation. Finally, a time-course study revealed a strong negative

correlation between the copy numbers of viral dsRNA and the developmental stages of the fungus.

**FEMS-1823**

**Poster Discussion: Veterinary Microbiology**

**CHARACTERIZATION OF VIRULENCE FACTORS AND IMMUNE-STIMULATING ANTIGENS IN *VIBRIO SALMONICIDA***

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**Background**

*Vibrio salmonicida* is the cause of cold-water vibriosis in farmed Atlantic salmon. The disease manifests as a hemorrhagic septicemia, with a mortality ranging from 3-90 %. In the 1980s, the disease was a major challenge to the Norwegian aquaculture industry, but it is now being effectively controlled by the use of oil-based vaccines. Nevertheless, in recent years there have been some reports of disease outbreaks. In 2013, 13 Norwegian fish farms were diagnosed with cold-water vibriosis, all localized in the three northernmost counties of Norway.

**Objectives**

The knowledge on virulence factors of *Vibrio salmonicida* and the subsequent immune response of the fish is limited. As bacteria adapt to their current environment, a bacterium isolated from a diseased fish will not be identical to the same species cultivated *in vitro*. The aim of this study was to explore the phenotype of bacteria present *in vivo*.

**Methods**

We have grown *Vibrio salmonicida* in semi-permeable implants in live fish and analyzed bacterial protein expression by two-dimensional gel electrophoresis and tandem mass spectrometry. The experiment was approved by the Norwegian Animal Research Authority (approval no. ID6228).

**Conclusions**

Our findings may contribute to the knowledge on disease progression in cold-water vibriosis, as well as to provide novel targets for more effective vaccines. Also, the



study may provide insights in the course of disease for other bacterial infections in fish.

**FEMS-0968**

**Poster Discussion: Veterinary Microbiology**

## **IDENTIFICATION AND TYPING OF LACTOCOCCUS GARVIEAE BASED ON MALDI TOF MS**

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### **Background**

MALDI-TOF-mass spectrometry (MS) has emerged as a powerful technique for the routine identification of microorganisms in clinical microbiology laboratories allowing an easier and faster diagnosis than conventional phenotypic and molecular methods. We have evaluated the suitability of MALDI-TOF for the rapid identification of *Lactococcus garvieae* the etiological agent of lactococcosis, a septicemic infection affecting different wild and farmed fish species, and recognized also as an opportunistic emerging human pathogen.

### **Objectives**

Assessment of the reliability of MALDI-TOF MS for the identification of *Lactococcus garvieae*

### **Methods**

A total of 35 isolates from different host and environments were obtained from the culture collection of VISAVET were used in the study. The identification results obtained by MALDI TOF were compared with those obtained by biochemical identification (API 32 Strep) and by a species- specific PCR assay (Zlotkin et al. 1998. J Clin Microbiol 36, 983-985). Mass spectra acquisition and analysis was performed on a Bruker UltraFlex platform (Bruker Daltonics) using MALDI Biotyper™ 3.0 software in the automatic mode using a matrix of saturated solution of  $\alpha$ -HCCA. In addition, a subset of isolates was subjected to PFGE.

### **Conclusions**

The majority of peaks were obtained in the range from m/z 2000 to 10000. The proteomic results matched with those of genotypic approach. Our results also showed some differences in MS spectra in *L. garvieae* isolates recovered from different origins/hosts suggesting the possible identification of molecular biomarkers for *L. garvieae*. Our study demonstrates that proteomics identification using MALDI TOF MS could be a reliable approach for identifying and discriminating this microorganism.



**FEMS-1160**

**Poster Discussion: Veterinary Microbiology**

**O SEROGROUPS OF ENTEROPATHOGENIC (EPEC) AND SHIGATOXIGENIC (STEC) ESCHERICHIA COLI FROM**

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**Background**

*Escherichia coli* producing the attachment-effacement (AE) lesion (EPEC) and/or Shiga toxins (STEC) cause enteritis and (bloody) diarrhoea in young calves and in humans, and are also present in the intestines of healthy cattle. Besides the O157:H7 serotype, EPEC and STEC can belong to more than sixty O serogroups. Of them, 8 have been most frequently identified worldwide: O5, O26, O103, O111, O118, O121, O145 and O165, with some also causing diarrhoea in young calves.

**Objectives**

This study aimed at identifying the pathotypes and the O serogroups of STEC and EPEC isolated from <1-month-old diarrhoeic calves in Wallonia, Belgium.

**Methods**

(i) 233 enterohaemolysin-producing *E. coli* were isolated at ARSIA between November 2008 and February 2014 from diarrhoeic calves after growth on EHLY Medium<sup>®</sup>. They were tested with a triplex PCR targeting the *stx1*, *stx2* (Shiga toxins) and *eae* (AE lesion) genes.

(ii) triplex PCR-positive *E. coli* were assayed with two pentaplex PCR targeting the specific genes coding for the nine O serogroups listed above and for the O104 serogroup.

**Conclusions**

(i) 206 isolates tested positive with the triplex PCR. The most frequent pathotypes were *eae+stx1+* (102 isolates), *eae+* (78 isolates) and *eae+stx1+stx2+* (13 isolates).

(ii) the most frequent serogroups of EPEC and STEC were O26 (57 isolates) and O111 (36 isolates). A few additional isolates tested positive for the O103, O5, O145, O121 and O157 serogroups.

(iii) the future is to compare these EPEC and STEC with those isolated from healthy cattle and from humans, to identify host- and age-specific properties.

FEMS-2576

Poster Discussion: Veterinary Microbiology

# CHARACTERISTICS AND ANTIMICROBIAL SUSCEPTIBILITY OF TRUEPERELLA PYOGENES ISOLATED FROM BOVINE MASTITIS CASES IN CHINA

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## Background

*Trueperella (T) pyogenes* is an opportunistic pathogen causes suppurative diseases in dairy cows. However, the pathogenesis and antibiotics resistance of *T. pyogenes* are still unclear.

## Objectives

*T. pyogenes* isolates morphological characteristics, the presence of genes (*plo*, *cbpA*, *fimA*, *fimC*, *fimE*, *fimG*, *nanH*, *nanP*, *tetW*, *ermX*, *ermB*), biofilm formation, the cytopathological effects in intracellular assay and antimicrobial resistance were investigated.

## Methods

*T. pyogenes* was isolated from 50 out of 275 clinical and subclinical bovine mastitis cases in China. Both pyolysin (*plo*) and collagen-binding protein (*cbpA*) virulence factor genes were detected by conventional PCR in all *T. pyogenes* isolates. The tissue culture plate method was used to assay the capacity of *T. pyogenes* for biofilm formation and showed that 90% of *T. pyogenes* isolates were able to form biofilms with different production amount. Minimum inhibitory concentrations (MICs) of 14 antimicrobial agents were determined and observed high susceptibility to rifampin (96%), while high resistance to trimethoprim–sulfamethoxazole (90%) and bacitracin (98%). Intracellular assay revealed that 4 different *T. pyogenes* isolates had different cytopathological effects on infected bovine mammary gland epithelial cells.

## Conclusions

18% *T. pyogenes* isolates indicates that *T. pyogenes* is important contributors to bovine mastitis. Moreover, the within-host quantitative, spatial, high occurrence of multidrug-resistant, biofilm producing and temporal dynamics of *T. pyogenes* interactions are key factors to better understand how immunity acts on infections with bacteria and how they evade immune surveillance; thus, highlighting the need for prudent use of antimicrobial agents in veterinary medicine (This research was supported by projects No. 2012BAD12B03, No. 313054, No. 20120008110042, No. 2014M561102 and No. GDT20141100043).

**FEMS-0643**

**Poster Discussion: Environmental Microbiology**

**FUNCTIONAL RESILIENCE OF MICROBIAL ECOSYSTEMS IN SOIL: HOW IMPORTANT IS A SPATIAL ANALYSIS?**

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**Background**

Microbial life in soil is exposed to fluctuating environmental conditions influencing the performance of ecosystem services such as biodegradation.

**Objectives**

However, as this environment is typically very heterogeneous, spatial aspects can be expected to play a major role for the ability to recover from a stress event.

**Methods**

To determine key processes for functional resilience, scenarios with varying stress intensities were simulated within a microbial simulation model and the biodegradation rate in the recovery phase measured. Besides temporal monitoring, results were analyzed regarding spatial and mechanistic aspects. Parameters including microbial growth and dispersal rates were varied over a typical range to consider microorganisms with varying properties.

**Conclusions**

Results of the mechanistic and spatial view show that key factors for functional recovery depend on stress intensity and the location of the observed habitats. The limiting factors near unstressed areas are spatial processes (motility and substrate diffusion), with increasing distance microbial growth becomes more important. To confirm this, we repeated the simulations including a dispersal network representing fungi in soil. The system benefits from an increased spatial performance due to higher bacterial mobility.

With these simulations we show the importance of spatial aspects even at the mm-scale for recovering after a severe stress event in a highly heterogeneous environment such as soil. In consequence a spatial-mechanistic view is necessary for examining the functional resilience as the temporal view alone could not have led to these conclusions.

Further research should explore the importance of a spatial view for quantifying functional resilience also after complex stress regimes.





**FEMS-1025**

**Poster Discussion: Environmental Microbiology**

**PHYSIOLOGIC AND GENOMIC CHARACTERIZATION OF A NOVEL NITROSPIRA SPECIES ENRICHED UNDER ANAEROBIC, DENITRIFYING CONDITIONS**

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**Background**

Members of the genus *Nitrospira* are dominant in many natural habitats and of vital importance for wastewater treatment. They are chemolithoautotrophic organisms capable of growth with nitrite and CO<sub>2</sub> as sole energy and carbon source. It further has been demonstrated that some *Nitrospira* can utilize simple organic carbon compounds and molecular hydrogen as alternative substrates and can switch to nitrate reduction under oxygen limitation. However, this metabolism has been assumed to be a survival strategy and in most systems studied so far *Nitrospira* was outcompeted by denitrifying organisms when anoxic conditions prevailed.

**Objectives**

Here, a community of nitrogen cycle bacteria was enriched from the anaerobic compartment of a biofilter connected to a recirculating aquaculture system.

**Methods**

The culture was fed with filtered water from the aquaculture system, supplemented with ammonium, nitrite and nitrate.

**Conclusions**

A stable enrichment culture was obtained which anaerobically converted ammonium, nitrite and nitrate into dinitrogen gas. The culture was dominated by anaerobic ammonium-oxidizing *Brocadia* species, a denitrifier (*Denitratisoma*), and a *Nitrospira* species. Anammox bacteria and *Nitrospira* co-aggregated, while the denitrifiers formed separate clusters. This culture shows that anaerobic ammonium oxidizers and nitrite oxidizers, who were considered to be mutually exclusive, can be grown as a stable co-culture. Furthermore, this shows that some *Nitrospira* species can be competitive under nitrate-reducing conditions. This study demonstrates another unexpected lifestyle for an organism believed to be only competitive under aerobic lithoautotrophic conditions. It further indicates that *Nitrospira* in combination with anammox can be of interest for anoxic wastewater treatment systems.

**FEMS-1295**

**Poster Discussion: Environmental Microbiology**

**BACTERIAL PROFILING OF SAHARAN DUST DEPOSITION IN THE ATLANTIC OCEAN USING SEDIMENT TRAP MOORINGS – YEAR ONE RESULTS**

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**Background**

Large quantities of dust are transported from the Sahara Desert across the Atlantic Ocean towards the Caribbean each year, with a large portion of it deposited in the ocean. This dust brings an array of minerals, nutrients and organic matter, both living and dead. This input potentially fertilizes phytoplankton growth, with resulting knock-on effects throughout the food chain. The input of terrestrial microbial life may also have an impact on the marine microbial community.

**Objectives**

The aim is to understand the links between dust input and the bacterial community and how this relates to ocean productivity and the carbon cycle.

**Methods**

The current multi-year project consists of a transect of floating dust collectors and sub-surface sediment traps placed at 12°N across the Atlantic Ocean. Sediment traps are located 1200m and 3500m below the sea surface and all are synchronized to collect samples for a period of two weeks.

**Conclusions**

The first set of sediment trap samples were recovered using the *RV Pelagia* in November 2013 with promising results. Results from 7 sediment traps (three at 1200m and four at 3500m) were obtained. In general, the total mass flux decreased as distance from the source increased and the upper traps generally held more material than those at 3500m. Denaturing Gradient Gel Electrophoresis (DGGE) was used as a screening technique, revealing highly varied profiles, with the upper (1200m) traps generally showing more variation throughout the year. Several samples have been submitted for high throughput DNA sequencing which will identify the variations in these samples.

**FEMS-2322**

**Poster Discussion: Environmental Microbiology**

**LIFE IN A MICROWAVE: EFFECTS OF MICROWAVE RADIATION ON THERMUS SP.**

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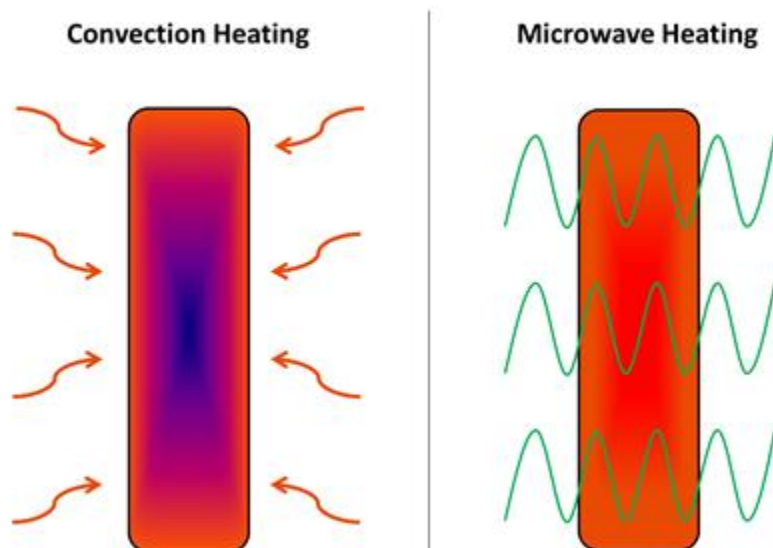
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**Background**

Biological systems are frequently exposed to microwave radiation. Many studies have investigated the influence of microwaves on these systems, but controversy over methods to distinguish between thermal and non-thermal microwave effects remains.



**Objectives**

To differentiate between non-thermal and thermal microwave effects on a physiology of a microorganism, a thermophilic bacterium was grown in a constant-temperature microwave or a convection oven. Comparing the growth properties of the thermophile in these conditions will reveal non-thermal microwave effects on cell growth and

physiology. Biophysical and biochemical analysis will demonstrate changes in morphology and chemical composition arising from microwave exposure.

### **Methods**

Cell growth was analyzed by optical density (OD) measurements (supported by independent quantitative DNA analysis), and cell morphologies were characterized using electron microscopy imaging (SEM, TEM), dynamic light scattering (DLS), and atomic force microscopy (AFM). AFM was also used to probe the biophysical characteristics of the cells, in conjunction with nano-infrared spectroscopy (Nano-IR). Attenuated total reflectance infrared spectroscopy (ATR-IR) and fatty acid methyl ester (FAMES) analysis were used to determine biochemical differences between cells grown in microwave and oven conditions.

### **Conclusions**

Thermophilic bacteria were grown in a synthetic microwave such that thermal effects and microwave effects were distinguishable. These data demonstrate that there are physiological differences between cells cultured in a dielectric field and a convection oven, and that microwaves induce non-thermal changes to the structure, physiology, and chemical composition of the organism.

**FEMS-2404**

**Poster Discussion: Environmental Microbiology**

**MYXOBACTERIA IN PEAT BOGS AND FEN – AN ASTONISHING DIVERSITY**

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**Background**

Increasingly appearing resistances to antibiotics and the subsequent need for new bioactive substances is one of the fundamental challenges of infection research. Myxobacteria have a rich secondary metabolism, which places them among the best known natural product producers. To date more than 100 new metabolites and more than 500 derivatives were described from these soil dwelling, predatory microorganisms. In the past it turns out that in particular new families, genera and species of myxobacteria are reliable sources for new compounds.

**Objectives**

Promising sources for the isolation of new myxobacteria are uncommon habitats like, for example, peat bogs or fen, which are characterized by low pH values. This habitat has been neglected with regard to the isolation of myxobacteria in the past. In our study, the diversity of myxobacteria in moor-samples from the Harz-region was evaluated by standard cultivation methods as well as by cultivation independent 16S rRNA-clone bank analyses.

**Methods**

A total of 297 clones of myxobacterial origin were sequenced and compared to sequences of cultures, isolated from these samples, by phylogenetic analyses. It turned out that the majority of myxobacteria is only represented by clone sequences and could not be cultivated. Comparing these sequences to sequences of a public database (NCBI) revealed that most of these uncultivated myxobacteria are exclusively related to other uncultivated myxobacterial sequences from acidic soils.

**Conclusions**

This suggests that peat bogs and fen harbor a big diversity of new myxobacterial species, genera and even families which themselves harbor a great potential of urgently needed, new secondary metabolites.

FEMS-1073

Poster Discussion: Environmental Microbiology

**THE MICROORGANISM THAT NEVER READ THE LITERATURE - FASTEST GROWING PHOTODAMAGE TOLERANT ALGA ISOLATED FROM DESERT CRUSTS, DEPENDS ON PIONEER FILAMENTOUS CYANOBACTERIA SPECIES TO SURVIVE DESICCATION**

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**Background**

With the premise that unique capabilities can be found in microorganisms facing extremely harsh conditions, we focused our efforts on desert biological sand crusts (BSC), one of the harshest environments to support life. Recently, we have isolated a small green alga (named *Chlorella ohadii*), which does not conform to some of the basic fundamentals in microbial ecology and photosynthesis:

**Objectives**

1. Acquired ability to acclimate to extreme environments is usually accompanied by reduced performance under optimal conditions. We intend to show that *C. ohadii* **does not obey this rule**. In its natural habitat, *C. ohadii* is facing diurnal desiccation-hydration cycles, vast temperature amplitudes and extremely high illumination intensities, yet when grown under optimal laboratory conditions it exhibits the fastest growth rates ever reported for an alga.
2. After many years of research, some feel that we have elucidated the functioning of the photosynthetic machinery, and what sets the upper limit for algal growth. However, the unparalleled fast growth, extremely high photosynthetic rates and resistance to photodamage, suggest this **may not be the case**. Namely, *C. ohadii* has been shown to be completely resistant to photoinhibition, and its productivity was unaffected by irradiances as high as twice full sun light.
3. Survival of *C. ohadii* in its BSC habitat depends on close association with filamentous cyanobacteria; **a unique and novel mode of interspecies interaction**.

**Methods**

We wish to present detailed physiological analysis of the unique properties supporting this phenomenal growth and resistances, combined with insights from their genomic, transcriptomic and metabolomic characterization.

**Conclusions**

Treves, H., Raanan, H., Finkel, O., Berkowicz, S., Keren, N., Kaplan, A. (2013) A newly isolated *Chlorella* sp. from desert sand crusts exhibits a unique resistance to excess light intensity. FEMS Microbiology Ecology. doi:10.1111/1574-6941

**FEMS-2437**

**Poster Discussion: Food Microbiology and Biotechnology**

**LEVANSUCRASE OF PSEUDOMONAS SYRINGAE AS PRODUCER OF NOVEL PREBIOTIC FRUCTANS: A STRUCTURE-FUNCTION STUDY OF THE ENZYME**

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**Background**

Gut microbiota has numerous roles in human life and its significance has thus far been underestimated. Therefore, more detailed studies on diversity and metabolism of gut microbiota are needed.

**Objectives**

Gut microbiota can be shaped by prebiotics. Levan-type ( $\beta$  2,6-linked) fructans can be considered as potential prebiotics<sup>[1]</sup>. We have synthesized levan and levan-type fructooligosaccharides by heterologously expressed levansucrase Lsc3 of *Pseudomonas syringae* pv. tomato. These substrates should affect gut microbiota as were perfectly fermented by a gut generalist *B. thetaiotaomicron*<sup>[2]</sup>. Here we focus on structure-function relationships of the Lsc3 protein and its biotechnological potential.

**Methods**

The His-tagged Lsc3 protein was site-directedly mutated, biochemical methods were used to characterize the mutant proteins and 3D structure modelling was applied to interpret the results.

**Conclusions**

Levansucrase Lsc3 has high polymerizing activity (up to 80%) and extremely high stability confirming its biotechnological potential for the synthesis of fructans. Several novel catalysis-related positions for levansucrases were revealed and their predicted location on a 3D model of Lsc3 will be presented.

*Acknowledgements:* This research was funded by the grant GLOMR9072 from Estonian Research Council and the ERF grant 3.2.0701.12–0041 (SLOMR12215T) managed by the Archimedes Foundation

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**FEMS-3150**

**Poster Discussion: Food Microbiology and Biotechnology**

**MATCHING GENOME AND TRANSCRIPTOME OF LACTOCOCCUS LACTIS STRAINS WITH ROBUSTNESS TOWARDS INDUSTRIAL STRESSES**

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**Background**

*Lactococcus lactis* is industrially employed to manufacture fermented dairy products. Spray drying is the cheapest preservation method for *L. lactis* starter cultures, but during this process cultures encounter heat and oxidative stress, resulting in low survival. Viability of starter cultures is essential for their role in dairy acidification, supporting the need to understand and improve their robustness.

**Objectives**

We have measured the ability of 39 *L. lactis* strains to survive industrially relevant stresses. This set of strains contained different *L. lactis* strains from dairy as well as plant origin. The observed 4-log variation in heat and oxidative stress survival was compared with genomic content, resulting in the identification of genes associated with robustness. Presence and activity of genes can play an important role in the observed diversity in robustness.

**Methods**

Therefore, four *L. lactis* strains with varying robustness phenotypes were fermented under twelve different conditions, varying in temperature, salt concentration, pH, and oxygen level. Cells were harvested at exponential phase of growth for transcriptome analysis and survival measurements.

**Conclusions**

The varying growth conditions resulted in up to 4-log differences in robustness towards heat and oxidative stress. Moreover, clear differences in gene expression profiles were observed. Correlation of robustness phenotypes and gene expression levels revealed transcriptome signatures for oxidative and heat stress survival. For strain MG1363 this included the *metC-cysK* operon, involved in methionine and cysteine metabolism, which triggered us to grow this strain in the absence of

cysteine, resulting in elevated expression levels of the *metC-cysK* operon and concomitant enhanced robustness towards oxidative stress.

**FEMS-0783**

**Poster Discussion: Food Microbiology and Biotechnology**

**COOPERATION BETWEEN SPECIES IN THE YOGURT CONSORTIUM IS  
AFFECTED BY THE MODULATION OF INTRACELLULAR PH THAT IS DRIVEN  
BY STREPTOCOCCUS THERMOPHILUS UREASE ACTIVITY**

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**Background**

The proto-cooperation between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in the yogurt consortium enhances the growth rate and size of each population. In contrast, the independent growth of the two species in milk leads to a slower growth rate and a smaller population size.

**Objectives**

In this study, we evaluated how urease activity of *S. thermophilus* might affect the the intracellular pH of *L. delbrueckii*, modulating its bioenergetics during milk fermentation.

**Methods**

Urease-mediated intracellular alkalization of *S. thermophilus* and *L. delbrueckii* was evaluated by flow cytometry using cFSE as pH-dependent fluorescent probe. Lactose consumption and lactic acid production was followed *in vivo* by <sup>13</sup>C-NMR analysis. D-L lactic acid production in milk was measured enzymatically. For the evaluation of the pH-dependent glycolysis efficiency, glucose and lactic acid were measured by HPLC.

**Conclusions**

We observed that intracellular alkalization caused by urea hydrolysis or the addition of ammonia to milk boosted lactic acid production in *S. thermophilus* and in *L. delbrueckii* when the species were grown separately or in combination. Therefore, we propose that urease activity acts as an altruistic cooperative trait, which is costly for urease-positive individuals but provides a local benefit because other individuals can take advantage of urease-modulated pH. Compared to the interactions that are known to occur between *S. thermophilus* and *L. delbrueckii* in the yogurt consortium, the modulation of the bioenergetic efficiency due to the intracellular pH alkalization represents a new type of cooperation that directly affects the kinetic parameters of enzymes involved in homolactic fermentation in both the species.



**FEMS-1827**

**Poster Discussion: Food Microbiology and Biotechnology**

**LISTERIA MONOCYTOGENES OF SEQUENCE TYPE 121 HARBOR SPECIFIC ADAPTATIONS SUPPORTING PERSISTENCE IN FOOD PRODUCTION PLANTS.**

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**Background**

The foodborne pathogen *Listeria monocytogenes* is able to survive for months and even years in food production environments. Among a great strain diversity particularly strains belonging to sequence type (ST)121 are persistent in food production plants.

**Objectives**

To elucidate the molecular determinants responsible for persistence of *L. monocytogenes*.

**Methods**

We analysed the genomes of two *L. monocytogenes* ST121 strains, which persisted for up to eight years in food production plants in Ireland and Austria. Additionally we characterized two ST121 strain-specific genetic features: Tn6188 and *lin0464/lin0465*.

**Conclusions**

All ST121 genomes are highly similar and show a tremendously high degree of conservation among prophages and particularly among their plasmids, which are usually variable parts of genetic information in bacteria. This remarkably high level of conservation suggests a strong selective pressure.

In addition, all ST121 strains share adaptations related to persistence in food production environments such as the presence of Tn6188, a transposon responsible for increased tolerance against quaternary ammonium compounds, and the presence of homologues of the *L. innocua* genes *lin0464* and *lin0465*, a transcriptional regulator and a putative pfpl protease. Deletion of *lin0465* resulted in reduced survival under oxidative and alkaline conditions suggesting a role in stress response. Furthermore all ST121 strains reveal a yet undescribed insertion harboring recombination hotspot (RHS) repeat proteins, which are most likely involved in

competition against other bacteria. In conclusion we show that *L. monocytogenes* ST121 strains are highly similar to each other harboring conserved regions which provide fitness adaptations to survival in food production environments.

**FEMS-2779**

**Poster Discussion: Food Microbiology and Biotechnology**

**USING DYNAMIC SINGLE CELL BIOLOGY TO DISSECT INJURY,  
RESUSCITATION AND DEATH PHENOMENA IN STRESSED POPULATIONS OF  
ESCHERICHIA COLI**

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**Background**

While inimical food preservation approaches often result in heavily damaged and stressed populations of foodborne pathogens and spoilage microorganisms, very little is known about the actual molecular and genetic events and dynamics that govern the (sub)lethal injury and subsequent resuscitation or death of these cells. Nevertheless, insights into these phenomena might be decisive for a proper understanding of the resulting behavior and evolvability of the surviving subpopulation.

**Objectives**

In this study, we set out to monitor and dissect (sub)lethal injury and resuscitation phenomena in populations and cells of *Escherichia coli* that are stressed by heat or high hydrostatic pressure treatment.

**Methods**

Directed evolution, genetics and time-lapse fluorescence microscopy.

**Conclusions**

Based on adaptive mutations, genetics and live cell biology, different subcellular processes and structures have been delineated and monitored that play a role in the heterogeneous injury and resuscitation dynamics of high temperature or pressure stressed *E. coli* cells and populations, thereby revealing a surprising impact on the spatial dynamics of nucleoids and protein aggregation inside stressed cells.

**FEMS-1788**

**Poster Discussion: Food Microbiology and Biotechnology**

**MAXIMIZING BIOTECHNOLOGICAL PRODUCTION OF 3-HYDROXYPROPIONALDEHYDE AS A NATURAL FOOD PRESERVATIVE WHILE MINIMIZING ITS CONVERSION TO TOXIC ACROLEIN**

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**Background**

3-hydroxypropionaldehyde (3-HPA) has strong antimicrobial activity and application potential as a natural food preservative for disinfection of minimally processed vegetables. During glycerol metabolism, *Lactobacillus reuteri* DSM 20016T can accumulate 3-HPA, which undergoes reversible dimerization and hydration, and can be further converted enzymatically into inactive 1,3-propanediol (1,3-PD), restoring intracellular redox potential. Dehydration of 3-HPA resulting in acrolein, a toxic metabolite, is also possible, however, conditions promoting this transformation have not been fully understood due to the lack of analytical methods to simultaneously quantify 3-HPA as well as acrolein.

**Objectives**

The aim of this study was to maximize the capacity for biotechnological processing of glycerol to 3-HPA using *L. reuteri*, while accounting for the accumulation of acrolein after production and storage to evaluate toxicological risk.

**Methods**

Strategies to improve 3-HPA production included the addition of redox-active compounds during glycerol fermentation to prevent conversion to 1,3-PD, the usage of immobilized cells resulting in high densities of viable cells as well as the optimization of process parameters in batch and fed-batch processes. Special attention was paid to cultivation conditions of *L. reuteri* prior to glycerol conversion. Acrolein and 3-HPA were simultaneously quantified using a newly established analytical method employing ion-chromatography with pulsed amperometric detection.

**Conclusions**

Redox-active compounds, process conditions and pre-treatment of *L. reuteri* cultures influenced 3-HPA yield as well as ratio of 3-HPA to 1,3-PD. Temperature and pH



optimization prevented formation of acrolein during biotechnological production and storage, promoting the safety of 3-HPA when applied as natural food preservative.

**FEMS-2440**

**Poster Discussion: Social interactions between microbes**

**CANDIDA GLABRATA BIOFILMS RESPONSE TO AMPHOTERICIN B**

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**Background**

*Candida* species are responsible for recurrent human infections, mostly in immunocompromised patients, due to their high vulnerability. *Candida glabrata* has been showing to have a major role in these infections being the second most prevalent species involved in human fungemia. Amphotericin B (AmB), a common antifungal drug, is a hospital-environment exclusive polyene, normally being efficient when used to fight candidiasis.

**Objectives**

The main goal of this work was to infer about the influence of AmB in *Candida glabrata* biofilms formation and its effect on matrix composition and *ERG* genes expression.

**Methods**

*Candida glabrata* biofilms were formed in the presence of AmB and analyzed by dry weight. Moreover, *ERG* genes expression was evaluated by qRT-PCR and matrix was analyzed in terms of composition in carbohydrates, proteins, beta-glucans and a new finding: ergosterol.

**Conclusions**

In addition to an inefficient reduction of the *C. glabrata* biofilms, this work showed that *ERG* genes seem to be less involved than the matrix composition in *C. glabrata* biofilms response to AmB. Specifically, *C. glabrata* biofilms matrices respond with an increase of carbohydrates, particularly beta-1,3 glucans, and with a decrease of total proteins. The ergosterol values did not expressively changed in the presence of AmB.

The present work support the theory of multifaceted mechanisms developed by *C. glabrata* biofilms as response to the presence of AmB.

**FEMS-1022**

**Poster Discussion: Social interactions between microbes**

**EFFECT OF SHEAR STRESS ON PSEUDOMONAS AERUGINOSA ISOLATED FROM THE CYSTIC FIBROSIS LUNG**

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**Background**

Chronic *Pseudomonas aeruginosa* lung infections are the main cause of morbidity in cystic fibrosis (CF) patients. Previously, we have sequenced the genome of the transmissible Belgian epidemic strain CF\_PA39. This CF-adapted strain has been present in the UZ Brussel hospital environment for at least ten years. Furthermore, earlier experiments showed that culturing *P. aeruginosa* PAO1 in a low fluid shear environment, obtained by means of the Rotating Wall Vessel (RWV) technology, leads to the formation of a biofilm phenotype comparable to that observed in the CF lung.

**Objectives**

To study the effect of fluid shear on biofilm formation by an adapted *P. aeruginosa* CF strain in artificial sputum medium (ASM) at the genotypic, transcriptomic, and phenotypic level.

**Methods**

In this study, an RWV experiment was performed that closely resembled the *in vivo* situation by inoculating this adapted CF isolate in ASM in the RWV either with or without two glass beads, simulating high and low fluid shear conditions, respectively. Scanning electron microscopy (SEM) was utilized to determine biofilm formation in both conditions, while RNAseq and qPCR was used to study gene expression in both conditions.

**Conclusions**

Increasing fluid shear in the RWV model disrupted biofilm formation of an adapted *P. aeruginosa* CF isolate.

In accordance with the biofilm disruption, several genes involved in denitrification, tryptophane synthesis, choline metabolism, and alginate biosynthesis were down-regulated in the high fluid shear condition, resembling the planktonic stage of growth. Furthermore, we identified small RNAs that are differentially expressed.

**FEMS-1357**

**Poster Discussion: Social interactions between microbes**

**CELL-CELL COMMUNICATION PROVIDES FITNESS BENEFITS TO POPULATIONS OF *LISTERIA MONOCYTOGENES* IN THE SOIL ENVIRONMENT**

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**Background**

*Listeria monocytogenes* is a ubiquitous opportunistic human pathogen detected in many habitats spanning from the farm environment to food industry and the gastrointestinal tract.

Cell-cell communication participates to the adaptation of bacteria to their environment. In the species *Listeria monocytogenes*, the Agr system is required for full virulence and biofilm formation but its actual role is still poorly understood.

**Objectives**

To investigate whether the ability to communicate provides a benefit to *L. monocytogenes* in soil, a complex environment combining biotic and abiotic characteristics.

**Methods**

Deletion of the gene coding the response regulator or the signal propeptide. Population dynamics of the mutant and/or parental strains in soil microcosms. Both unsterilised and sterilised soils were investigated.

**Conclusions**

Deletion of the gene coding the regulator or the signal did not affect population dynamics in sterilized soil but survival was altered in biotic soil suggesting that the Agr system was involved to face the complex soil biotic environment. This was confirmed by co-incubation experiments. The fitness of the response negative mutant was lower either in the presence or absence of the parental strain but the fitness of the signal negative mutant depended on the strain with which it was co-cultured. Survival of the signal negative mutant was higher when co-cultured with the parental strain than when co-cultured with the response negative mutant. These results showed that the ability to respond to communication provided a benefit to listerial cells. These results might also indicate that in soil, the Agr system controls private goods rather than public goods.



**FEMS-2645**

**Poster Discussion: Social interactions between microbes**

**REGULATORY RNA ARISES FROM 3'-UTR OF AHL SENSOR ENCODED RNA AFTER ITS PREPROCESSING IN PECTOBACTERIUM ATROSEPTICUM**

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**Background**

In a broad group of proteobacteria two quorum sensing-related genes, which encode synthase of autoinducers and their sensor, respectively, have convergent topology and overlap by their 3'-ends. It was suggested that the expression of one gene may antagonize the transcription of another one because of the convergent arrangement of these genes.

**Objectives**

We proposed that the topology of quorum sensing-related genes of plant pathogenic bacterium *Pectobacterium atrosepticum*, *expI* and *expR*, determines the mechanism of regulation of their expression.

**Methods**

We noticed that *expR* gene lacks any obvious transcriptional stop signals. Using chain-specific RT-qPCR we found that at the stationary growth phase *expR* mRNAs having long 3'-untranslated region (UTR) were formed and the expression of oppositely oriented *expI* gene was simultaneously decreased. It is likely, that RNA products of the *expR* regulatory gene may inhibit the expression of the autoinducer synthase gene through the interactions with *expI* mRNA. Using 3'-RACE method we found that at the stationary growth phase the extended *expR* transcripts underwent preprocessing resulting in their break down into two fragments. The first fragment encompassed the entire length of open reading frame (ORF) of *expR* gene and the second one included only a long 3'-UTR having a predicted regulatory function.

**Conclusions**

We found that *cis*-encoded RNA may participate in the regulation of expression of two convergent functionally related genes. The length of one of two transcripts varies respective to the cell growth phase affecting the expression of oppositely oriented gene. Moreover, *cis*-encoded regulatory RNAs arises from protein encoded RNAs that undergo preprocessing in their 3'-UTR.

**FEMS-2528**

**Poster Discussion: Social interactions between microbes**

**METABOLIC TRANSFORMATIONS ENHANCES PSEUDOMONAS AERUGINOSA GROWTH ACTIVITY DURING INTERACTION WITH STAPHYLOCOCCUS AUREUS**

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**Background**

Interactions among members of polymicrobial infections can result in altered pathogen behaviours such as enhanced virulence, biofilm formation or antibiotic tolerance, which may influence the disease phenotype and clinical outcome of the infection. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are important opportunistic human pathogens and are both part of the polymicrobial infection communities in human hosts. Cell-cell communication can play a major role in the interaction behaviors between these bacteria, which subsequently can affect the physiology of the individual strains. However, the extent to which evolutionary processes may remodel interspecies interactions and bacterial physiology during the course of infection and therapy is currently not understood.

**Objectives**

Therefore, the aim of this study was to analyze *in vitro* interactions between *S. aureus* and a human host adapted *P. aeruginosa* strain from a dominant lineage, DK2, that have evolved through decades of growth in chronically infected patients.

**Methods**

By using a combination of *in vitro* agar assays, molecular genetics and MALDI-TOF imaging mass spectrometry we demonstrate that DK2 – in contrast to other *P. aeruginosa* strains - show a commensal-like interaction with *S. aureus* during *in vitro* co-culturing, where the growth activity of *P. aeruginosa* was increased in the presence of *S. aureus*. The commensal interaction was observed both under aerobic and anaerobic conditions as well as during growth on artificial CF sputum medium, however was abolished with *S. aureus* strains mutated in the *agr* quorum sensing system.

**Conclusions**

Our results suggest that metabolic transformations during interaction with *S. aureus* enhance the growth activity of *P. aeruginosa*.





**FEMS-2613**

**Poster Discussion: Social interactions between microbes**

**ADVANCEMENTS TOWARDS A SYSTEM-LEVEL UNDERSTANDING OF SUBAERIAL BIOFILMS INHABITING OUTDOOR STONE MATERIALS**

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**Background**

Many of the world's most precious artworks are made of stone. Their irreversible deterioration due to biological attack is a worldwide concern. Microorganisms colonize outdoor lithic surfaces and develop into biofilms at the interface solid/air (subaerial biofilms, SABs), which, in turn might cause aesthetic, chemical and physical decay. Although it has been estimated that at least 99% of the world's microbial biomass exists in biofilms, the role and behavior of microorganisms within the biofilm matrix and their complex interactions with the external environment is still unknown.

**Objectives**

This work provides a pioneering and multidisciplinary research to investigate the behavior of microorganisms within the biofilm matrix for sorting out time-spatial relationships and to elucidate microorganism-EPS, inter-organism, biofilm-atmosphere and biofilm-stone interactions.

**Methods**

This work spans sophisticated molecular, chemical, physical and data modeling techniques and it is approached from two complementary angles:

- 1- Lab-scale study to delineate specific transcriptional responses of mono- and multi-species biofilms as well as the biofilm-stone interactions under controlled environmental conditions.
- 2- Real heritage case studies to investigate the shifts in the microbial community structure and function under different environmental conditions. Through comparing phylogenetic and functional diversity under different environmental scenarios, we

provide evidence that any intuition gained from the lab-scale experiments is relevant to true environmental biofilms.

## **Conclusions**

The findings obtained so far will contribute to better understand the complexity of all the interactions encountered within SAB communities, and how these interactions may influence the biofilm outcome and the biodeterioration of the stone materials under different environmental conditions.

**FEMS-2136**

**Anaerobic physiology**

**NITRIC OXIDE CONCENTRATION AND BET-HEDGING DURING THE OXIC-ANOXIC TRANSITION IN PARACOCCLUS DENITRIFICANS**

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**Background**

The reduction of nitrite to NO is considered the defining step of denitrification and a point of no return due to the toxicity of NO. Nevertheless, some accumulation of NO is necessary since it is central to the induction of its reductase and frequently also its enzymatic origin. A positive feedback loop perpetuated by a toxic substance is intuitively a play with fire. However, phenotypic studies of organisms known, or assumed to hold a product induced nitrite reductase have with few exceptions revealed stringent NO control.

**Objectives**

We studied the gas kinetics of *P. denitrificans* during the oxic-anoxic transition in order to reveal new aspects of NO as a regulator of denitrification.

**Methods**

The core instrumentation was a semi-automatic incubation system similar to that described by Molstad *et al.* (2007), where relevant headspace gases are monitored in respiring batch cultures.

**Conclusions**

In cultures incubated at 20°C, [NO] reached a semi-steady state at ~15nM. This homeostasis could almost entirely be explained by an estimated *in vivo* K<sub>m</sub> of 50nM for NO reductase. Although assumedly eliminating toxic effects, upholding such low [NO] appears to have some drawbacks. By combining experimental data with modelling approaches, we found strong evidence that the gross majority of cells were essentially entrapped in anoxia due to delayed induction of nitrite reductase. In contrast, when incubated at 30°C, [NO] was elevated (~75nM) and the entire population apparently succeeded in switching to N-oxide reduction. This illustrates some of the challenges of wielding a substance with such a double-edged nature: toxic intermediate and essential regulator in one.

**FEMS-2572**

**Anaerobic physiology**

**METHANOL AS A SUBSTRATE FOR METHANOGENESIS OF THE PRINCIPAL HUMAN GUT ARCHAEAL COMMENSAL METHANOBREVIBACTER SMITHII**

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**Background**

*Methanobrevibacter smithii* (*Mb. smithii*) is the predominant methanogen of the human gut microbiota using CO<sub>2</sub> and H<sub>2</sub> (or formate) for methanogenesis. Other gut methanogens utilize methanol (*Methanosphaera stadtmanae*) and methylamines (members of newly identified order Methanomassiliicoccales) by H<sub>2</sub>-dependent methanogenesis.

**Objectives**

Genomic analysis revealed that genes encoding the methanol-utilizing methanogenesis pathway (*mtaABC*) are present in most of the human gut strains of *Mb. smithii*, encompassing the completely sequenced DSMZ 861 strain. This arises the possibility that this methanobacteriale is also able to use methanol as substrate in the gut. This hypothesis was therefore tested.

**Methods**

The reference strain DSMZ 861 (ATCC 35061) was obtained from DSMZ (Braunschweig, Germany) and was cultured in DSM141 media under different conditions regards to H<sub>2</sub>, CO<sub>2</sub> and methanol. Gene expression of *mtaB* and *mcrA* genes (involved respectively in the methanol use and in the final common step of the methanogenesis pathways) were quantitatively evaluated by qPCR.

**Conclusions**

The DSMZ 861 strain originating from the human gut is able to perform methanogenesis with methanol and H<sub>2</sub>. A lower methane production rate was recorded when compared to normal hydrogenotrophic pathway. Methanol induces the expression of the *mtaB* gene while *mcrA* relative expression was lowered. This capability to use methanol coupled with its standard hydrogenotrophic methanogenesis provides a clue to explain the predominance of *Mb. smithii* over other methanogens in the human gut.

**FEMS-0849**

**Anaerobic physiology**

**SIGMA54-DEPENDENT REGULATION OF P-ETHYLPHENOL AND P-HYDROXYACETOPHENONE DEGRADATION IN “AROMATOLEUM AROMATICUM” EBN1**

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**Background**

Anaerobic *p*-ethylphenol and *p*-hydroxyacetophenone degradation in the denitrifying bacterium “*Aromatoleum aromaticum*” EbN1 is predicted to be transcriptionally controlled by the sigma54-dependent regulator EbA324. The latter is encoded in between the two operons comprising genes of *p*-ethylphenol/*p*-hydroxyacetophenone degradation and detoxification.

**Objectives**

Generation of an unmarked, *in-frame* deletion mutant (strain EbN1  $\Delta$ ebA324) to validate EbA324 as mediator of substrate-specific transcriptional activation.

**Methods**

The established genetic system for strain EbN1 was applied to generate deletion mutant strain  $\Delta$ ebA324 and the corresponding complementation strain  $\Delta$ ebA324::*ebA324*. Both mutant strains and the wild type were physiologically analyzed applying *p*-ethylphenol, *p*-hydroxyacetophenone, benzoate or a mixture of benzoate- *p*-hydroxyacetophenone and subsequently studied on the transcript and protein level.

**Conclusions**

The  $\Delta$ ebA324 mutant was unable to grow with both *p*-ethylphenol and *p*-hydroxyacetophenone. Accordingly, no transcripts of the *p*-ethylphenol operons were detected in the  $\Delta$ ebA324 mutant using benzoate as growth promoting co-substrate. In contrast, transcripts and proteins of both operons were detected in the wild type and complemented mutant strain, supporting the proposed function of EbA324. Furthermore, transcriptional activation by EbA324 depends on the presence of either *p*-ethylphenol or *p*-hydroxy-acetophenone: high level constitutive formation of EbA324 in the complemented mutant only yields operon expression in the presence of these compounds.

**FEMS-1573**

**Anaerobic physiology**

## **ADAPTATION OF LISTERIA WEIHENSTEPHANENSIS TO ANAEROBIOSIS**

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### **Background**

*Listeria weihenstephanensis* is a recently new described *Listeria* specie. It was isolated from the water plant *Lemna trisulca* from a fresh water pond in Bavaria, Germany. The isolate is non-haemolytic and has not been associated with animal or human diseases. It has an optimal growth at pH 7-8 and temperature at 28-34 °C. It requires a relative low demand of nutrients and is able to grow under both aerobic and anaerobic conditions.

### **Objectives**

In the present study, the focus was the investigation of the adaptation of *L. weihenstephanensis* to oxygen availability, with respect to the temperature-dependent influence.

### **Methods**

Growth analyses for *L. weihenstephanensis* were performed aerobically and anaerobically in BHI medium at 18 °C and 34 °C. Global transcriptional analyses, via next generation RNA sequencing, were then performed for cells grown aerobically or anaerobically to an OD<sub>600</sub> = 0.85-0.90. The oxygen dependent gene expression of selected genes was further validated via qPCR.

### **Conclusions**

At 18 °C, 49 genes were found to be stronger transcribed aerobically while 40 genes were found to be stronger transcribed anaerobically. At 34 °C, 52 genes exhibited aerobically a stronger transcription while transcription of 27 genes was induced anaerobically. Among the differently regulated genes, many encode for metabolic enzymes indicating broad metabolic adaptations with respect to oxygen availability. Moreover, the transcriptional results clarified that temperature does not play a key role in this oxygen-dependent regulation for *L. weihenstephanensis*.



**FEMS-1605**

**Anaerobic physiology**

**C<sub>4</sub>-DICARBOXYLATE TRANSPORT AND GLOBAL TRANSCRIPTOME ANALYSIS OF C<sub>4</sub>-DICARBOXYLATE METABOLISM IN ACTINOBACILLUS SUCCINOGENES**

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**Background**

*Actinobacillus succinogenes* that is known to produce large amounts of succinate during hexose fermentation was able to grow on C<sub>4</sub>-dicarboxylates like fumarate under aerobic and anaerobic condition. Succinate was the major product from anaerobic growth on fumarate, similar to growth on glucose. It indicates the involvement of fumarate respiration and the presence of proteins for C<sub>4</sub>-dicarboxylate transport.

**Objectives**

This study aims to characterize transport properties related to C<sub>4</sub>-dicarboxylate metabolism of *A. succinogenes*.

**Methods**

Transport of <sup>14</sup>C-fumarate and <sup>14</sup>C-succinate were studied with bacterial cells of *A. succinogenes* grown on fumarate under anaerobic condition. Transport properties were determined concentration-, time-, pH-dependently or in the presence of ionophores.

Global transcriptome was analyzed by total-RNA-sequencing using MiSeq platform. The total RNAs were isolated from cells grown on fumarate or glucose under aerobic or anaerobic condition.

**Conclusions**

Transport assay revealed that *A. succinogenes* has several transport systems representing specific uptake activities dependent on concentration range of for C<sub>4</sub>-dicarboxylates. Some of them required proton potential, but the others Na<sup>+</sup> gradient.



The genome of *A. succinogenes* possesses candidate genes encoding 18 different transport systems. Global transcriptome analysis showed that six transport systems are expressed during anaerobic growth on fumarate, which includes transporters of the Dcu, DcuC, DASS, and TRAP family.

Overall, *A. succinogenes* could utilize six different protein systems for anaerobic C<sub>4</sub>-dicarboxylates transport, according to availability of driving force and substrate concentration.

**FEMS-1668**

**Anaerobic physiology**

## **AN UNUSUAL METHANOGENIC PATHWAY FOUND IN METHANOMASSILIICOCCUS LUMINYENSIS**

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### **Background**

*Methanomassiliicoccus (Mmc.) luminyensis* is a methanogenic archaeon and was first isolated from human feces. The organism belongs to the recently discovered order of the Methanomassiliicoccales and uses methylated compounds together with H<sub>2</sub> as substrates, which is distinct from other methanogenic pathways.

### **Objectives**

*Mmc. luminyensis* shows unique metabolic features since it combines different methanogenic strategies in the course of heterodisulfide reduction possessing both, a soluble heterodisulfide reductase (HdrABC) and HdrD as compound of the membrane bound heterodisulfide reductase found in members of the order Methanosarcinales. Therefore the heterodisulfide reduction system of *Mmc. luminyensis* is of great scientific interest.

### **Methods**

In a novel approach using quantitative reverse transcriptase PCR we identified key enzymes of the methanogenic pathway of *Mmc. luminyensis*. To further investigate the role of membrane bound enzymes during methanogenesis membranes of *Mmc. luminyensis* were isolated and tested for the oxidation or reduction of artificial electron mediators such as viologen dyes. Additionally enzymes from *Mmc. luminyensis* will be produced heterologously and characterized in order to elucidate the methanogenic metabolism of the organism in detail. A special focus will be placed on the headless F<sub>420</sub>H<sub>2</sub> dehydrogenase (Fpo) complex lacking subunit FpoF, which is essential for F<sub>420</sub>H<sub>2</sub> oxidation, and HdrD since the HdrE subunit of the usually membrane bound heterodisulfide reductase is missing.

### **Conclusions**

If those enzymes are active despite their deficiency of certain key subunits *Mmc. luminyensis* could employ a new methanogenic pathway combining classical enzymes known from other methanogens in a so far not described manner.



**FEMS-2003**

**Anaerobic physiology**

**REDUCTIVE DECHLORINATION OF EITHER TETRACHLOROETHENE OR 1,2-DICHLOROETHANE BY DEHALOGENASES WITH SIMILAR PROPERTIES BUT DIFFERENT REACTION MECHANISMS**

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**Background**

Organohalide respiring bacteria are able to use halogenated organic compounds as terminal electron acceptors for growth. The key enzyme for the conversion of these substrates is the reductive dehalogenase (RDase), a cobamide-containing iron-sulfur protein. Biochemical and structural data showed the cobamide cofactor as the reactive species in the RDase's active site [1]. Numerous RDase protein sequences are listed in databases [2], but only a few enzymes were biochemically characterized. The RDase of *Sulfurospirillum multivorans*, PceA, converts tetrachloroethene via trichloroethene to *cis*-1,2-dichloroethene in a hydrogenolysis reaction [3]. In contrast, the RDase of *Desulfitobacterium dichloroeliminans*, DcaA, catalyzes the formation of ethene from 1,2-dichloroethane by a dihaloelimination mechanism [4].

**Objectives**

The structural determinants in RDases will be identified, which are responsible for the catalysis of either a hydrogenolysis or a dihaloelimination reaction.

**Methods**

In silico structural analysis of DcaA based on the crystal structure of PceA is conducted. The substrate spectrum of recombinant DcaA is determined via enzyme activity measurements. Site-directed mutagenesis is applied to unravel the role of amino acids forming the DcaA's active site.

**Conclusions**

From the results of this study, a better understanding of the diversity in RDase substrate spectra and reaction mechanisms and an improved classification of unknown RDases based on their protein sequence is expected.

[1] Bommer *et al.* (2014). Science 346: 455-458

[2] Hug *et al.* (2013). Phil Trans R Soc B Biol Sci 368: 20120322

[3] Neumann *et al.* (1995). Arch Microbiol 163: 276-281

[4] De Wildeman *et al.* (2003). Appl Environ Microbiol 69: 5643-5647

**FEMS-0785**

**Anaerobic physiology**

## **THE ANAEROBIC LINALOOL METABOLISM OF THAUERA LINALOOLENTIS 47LOL**

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### **Background**

*Thauera linaloolentis* 47Lol<sup>T</sup> grows on linalool (3,7-dimethylocta-1,6-dien-3-ol), a tertiary monoterpene alcohol, as sole carbon and energy source under denitrifying conditions. Linalool mineralization is initiated by isomerization into the primary alcohol geraniol by a novel enzyme, a linalool isomerase (Foss and Harder, 1997, Foss and Harder, 1998). Geraniol degradation may proceed in analogy to the acyclic terpene utilization (ATU) pathway, as described for various *Pseudomonas* strains (Förster-Fromme and Jendrosseck, 2010). This pathway involves an oxidation of geraniol to geranic acid. After activation by coenzyme A thioester formation, the monoterpene structure is shortened by beta-oxidation, and rearrangement to 3-methylcrotonyl-CoA, ultimately entering the leucine degradation pathway (LIU).

### **Objectives**

The objective of this study was to elucidate the anaerobic linalool metabolism in *Thauera linaloolentis* 47Lol.

### **Methods**

Recently, draft genomes of *T. linaloolentis* 47Lol became available and we detected candidate genes of the ATU and LIU pathways in the genome. Expressed proteins present in linalool-grown cells included many corresponding proteins.

### **Conclusions**

So far, the ATU pathway has only been described in various *Pseudomonas* strains growing aerobically. Our findings are the first observation for the utilization of the ATU pathway under anaerobic conditions.

**FEMS-2183**

**Anaerobic physiology**

**BONCAT TAGGING FOR DIRECT QUANTIFICATION OF RESPIRING AND NON-RESPIRING SUBPOPULATIONS IN PARACOCCLUS DENITRIFICANS DURING TRANSITIONS FROM AEROBIC RESPIRATION TO DENITRIFICATION**

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**Background**

Ecophysiological studies have revealed that the regulation of denitrification pathways is highly variable among bacterial strains. One aspect of the regulatory phenotype is the maintenance and distribution of the denitrification proteome within clonal populations. Gas kinetics studies of batch cultures during the transition from aerobic respiration to denitrification have indicated that in many organisms there is a uniform onset of N-oxide reduction amongst cells in the population. Mathematical modelling suggests that the model organism for denitrification studies, *Paracoccus denitrificans*, has a different strategy reminiscent of bet-hedging where only a subset of cells (Fden) start to denitrify, and thus continue to grow, upon oxygen depletion [1].

**Objectives**

We aim to estimate the proportion of cells successfully switching to denitrification by quantifying directly the cells that are showing translational activity after the onset of denitrifying conditions.

**Methods**

The bioorthogonal noncanonical amino acid tagging (BONCAT) approach takes advantage of chemical groups that react with each other at near-physiological conditions, but not with other cell components. These are appended to the bacterial proteome during translation using supplementation of a noncanonical amino acid, in this case azidohomoalanine (AHA). A fluorescent dye displaying a complementary moiety is then used to visualize cells displaying the modified proteins.

**Conclusions**

The BONCAT method was shown to be successful in differentiating between fully respiring cells and those that were inactive, only showing low background fluorescence. Studies are currently undertaken to determine Fden for *P. denitrificans* cultures undergoing the transition from aerobic to denitrifying growth.

[1] Hassan, J., Bergaust, L.L., Wheat, I.D., & Bakken, L.R. (2014). PLoS computational biology, 10(11), : 10.1371/journal.pcbi.1003933

**FEMS-2269**

**Anaerobic physiology**

## **A NOVEL ANAEROBIC TOLUENE-DEGRADING BACTERIAL STRAIN OF CLOSTRIDIACEAE FROM AN OIL-CONTAMINATED SOIL**

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### **Background**

Anaerobic degradation of aromatic hydrocarbons is important for bioremediation of contaminated subsurface environments. Information on the microbial diversity and metabolic pathways involved in anaerobic degradation of hydrocarbons is crucial for successful cleanup of hydrocarbons in subsurface. Although members of the family *Clostridiaceae* have been frequently observed in the analysis of various hydrocarbon-contaminated microbial communities, there are limited studies on the biodegradation potential of *Clostridiaceae*.

### **Objectives**

The aims of this study are to isolate hydrocarbon degrading strains of *Clostridiaceae* and elucidate the biochemical and genomic properties of the strain regarding anaerobic biodegradation.

### **Methods**

Enrichment cultures inoculated with various soils from oil spill sites were incubated for several years with petroleum amendment. After enrichment, several colonies were obtained using the roll tube method. Among the isolates, one strain, designated IRF9, was found to degrade toluene and thus selected for further biochemical and genomic analysis.

### **Conclusions**

An alkaliphilic and heterotrophic aromatic hydrocarbon-degrading bacterial strain, IRF9, was isolated from the enrichment cultures. Cells of strain IRF9 were straight or curved rod-shaped, motile, and Gram-negative. Optimal growth of strain IRF9 was observed at pH 9.0–9.5 and 40 °C. The strain was found to grow within pH and temperature ranges of 6.5–10.0 and 25–45 °C, respectively. Ferric iron was used as an electron acceptor but not sulfate, nitrate. The biochemical and genomic studies of the strain IRF9 will shed light on the potential of *Clostridiaceae* for the anaerobic biodegradation of hydrocarbons in subsurface environments.

**FEMS-1264**

**Anaerobic physiology**

**HUPO PLAYS IMPORTANT ROLE IN THE REGULATION OF THE HUPSL  
HYDROGENASE OF THIOCAPSA ROSEOPERSICINA**

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**Background**

The photosynthetic purple sulphur bacterium, *Thiocapsa roseopersicina* harbours four functional [NiFe] hydrogenases. Two of them are attached to the periplasmic membrane (HynSL, HupSL) and the other two are localized in the cytoplasm (Hox1, Hox2). It prefers to utilize reduced sulphur compounds for anaerobic phototrophic growth.

**Objectives**

HupSL is a hydrogen uptake hydrogenase which can recycle hydrogen in the environment and provides energy for the cells. The gene expression and enzyme activity correlate with the applied thiosulphate concentration, HupSL shows increased hydrogen uptake activity under low sodium-thiosulphate concentration. The hydrogenase small and large subunits are encoded by the *hupS* and *hupL* genes in the *hupSLCDHIOR* operon. The function of *hupO* gene and its product was completely unknown. In order to investigate the physiological role of the HupO, the *hupO* gene was inactivated by in-frame deletion mutagenesis. The *hupO* gene was deleted in the  $\Delta hynSL$ ,  $\Delta hox1H$ ,  $\Delta hox2H$  (GB113141) mutant (resulting strain HOD134). The effects of the mutation were analyzed by activity and expression measurements.

**Methods**

The *in vivo* activity of the HupSL hydrogenase in the HOD134 strain substantially increased under low sodium thiosulphate concentration compared to the wild-type and complemented strains. These activity measurements were supported by Western analyses and gene expression studies.

**Conclusions**

This study adds to the clarification of the so far unresolved HupSL regulation. The HupO plays a crucial role in this regulation at least under low thiosulphate conditions, where the masking effect of thiosulphate is avoided.



**FEMS-1064**

**Anaerobic physiology**

**EFFECT OF CARBON DIOXIDE ON EXOPOLYSACCHARIDE PRODUCTION AND SURVIVAL OF BIFIDOBACTERIUM BIFIDUM AFTER FREEZE-DRYING**

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**Background**

*Bifidobacteria* are particularly sensitive to dissolved oxygen potentially present in bioreactor during the production process [1]. However, oxygen stripping by another gas (i.e., nitrogen and hydrogen) decreases the oxido-reductive potential and improved survival of *B. bifidum* during storage [2]. It is noted that carbon dioxide can not only maintain the anaerobic growth conditions of *Bifidobacterium longum* JBL05 but also enhance the cell concentrations and the exopolysaccharide (EPS) secretion [1]. Moreover, EPS was known to be an important factor for the protection of *B. bifidum* during freeze-drying [3].

**Objectives**

Optimization of oxygen stripping by using different gases (i.e., CO<sub>2</sub> and N<sub>2</sub>) to reduce the redox potential in *B. bifidum* THT0101 culture aimed at (i) improving the survival of cells during freeze-drying and (ii) increasing the EPS secretion during the fermentation.

**Methods**

*B. bifidum* THT0101 was cultured following different degassing procedures. After culturing and freeze-drying, the concentration of exopolysaccharide and the viability of *B. bifidum* THT0101 were evaluated.

The results pointed out a significant increase of viability (5.06-15.6 folds by comparison with controls) in relation with the duration of degassing by CO<sub>2</sub>. Besides, the CO<sub>2</sub> treatments led to a decrease of the redox potential. The concentration of exopolysaccharide increased related to the flow rate of CO<sub>2</sub> and *B. bifidum* THT 0101 survival.

**Conclusions**

The flow rate of CO<sub>2</sub> in culture led to the increase of exopolysaccharide concentration and *B. bifidum* THT0101 survival.

**FEMS-0829**

**Anaerobic physiology**

**TRANSPOSON MUTANTS AFFECTING THE METABOLISM OF BICYCLIC MONOTERPENES IN *C. DEFRAGANS* 65PHEN**

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**Background**

Terpenes are natural hydrocarbons mainly produced by plants. Monoterpenes are released to the environment in teragram amounts every year. *Castellaniella defragans* 65Phen, a betaproteobacterium able to grow under denitrifying conditions, uses several aliphatic and cyclic monoterpenes as sole carbon and energy sources (1). This metabolic capability is currently studied intensively and the genome has recently been described (2).

**Objectives**

Identify genes and proteins relevant to the metabolism of the bicyclic monoterpenes 3-carene, sabinene and alpha-pinene.

**Methods**

Random transposon mutagenesis and differential proteomics were used.

**Conclusions**

Firstly, in our study the bicyclic monoterpenes 3-carene, sabinene and alpha-pinene seemed to be isomerized to monocyclic monoterpenes and then further degraded by proteins encoded in a 70 kb genetic island (2). The nucleotide composition of the island is indicative for horizontal gene transfer from several other hydrocarbon degraders to *C. defragans* 65Phen (2). Beta-oxidation of perillic acid and the leucine/isovalerate (liu) pathway were also involved in the metabolic steps of all monoterpenes under investigation. Additionally, several permeases and stress-response proteins had a relevant role in the survival and growth of *C. defragans* 65Phen on monoterpenes, most likely due to the monoterpenes toxicity.

1. Foss, S *et al.* *Systematic and Applied Microbiology*, 1998, **21**:237.
2. Petasch, J *et al.* *BMC Microbiology*, 2014, **14**:164.

**FEMS-1146**

**Anaerobic physiology**

**NOVEL LINEAGE OF VERSATILE SULFATE REDUCERS PROMOTES  
MICROBIAL NICHES IN ACIDIC CONDITIONS**

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**Background**

Generally, sulfate-reducing bacteria (SRB) grow optimally at neutral pH but sulfate reduction at low pH has been reported in both natural and engineered environments. Despite evidence of their activity *in situ*, acidophilic SRB have been difficult to isolate and cultivate *in vitro*, possibly due to the inhibitory effect at low pH of metabolites such as sulfide or acetic acid. Thus far, only three species have been formally described: *Desulfosporosinus acidiphilus*, *D. acididurans* and *Thermodesulfobium narugense*.

**Objectives**

Our objective was to isolate novel acidophilic SRB to study their ecophysiology.

**Methods**

Enrichments were set up using sediments from an acid rock drainage environment (Tinto River) and several isolation methods were applied (enrichment, serial dilution and pasteurization).

**Conclusions**

The isolate, strain I, represents a novel lineage of SRB within the *Firmicutes* phylum, proposed as *Desulfobacillus* spp. (93% similarity with the genera *Desulfosporosinus* and *Desulfitobacterium*). The genome-guided characterization shows a very versatile metabolism; it is able to use multiple electron donors and electron acceptors for respiratory growth, and to perform fermentation and disproportionation. Strain I shows a unique metabolism compared to other acidophilic SRB by being able to oxidize acetic acid. As a consequence, strain I is the most acidophilic strain described so far, able to grow while reducing sulfate at a pH as low as 3.8 (optimum at pH 5).

Its high tolerance to low pH and its acetate detoxifying metabolism together with the alkalinity generation coupled to growth of SRB suggest its important role in promoting microbial niches with milder conditions in acidic environments.



**FEMS-1942**

**Anaerobic physiology**

## **CONVERSION OF CIS-2-CARBOXYCYCLOHEXYLACETIC ACID IN THE DOWN-STREAM PATHWAY OF ANAEROBIC NAPHTHALENE DEGRADATION**

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### **Background**

The ability of anaerobic naphthalene degradation has been demonstrated for the *deltaproteobacterial* strains N47 and NaphS2 under sulfate-reducing conditions (Galushko *et al.*, 1999, Meckenstock *et al.*, 2000). It was shown recently that after carboxylation to 2-naphthoic acid (Moultaki *et al.*, 2012) and formation of the corresponding CoA-ester, the latter is stepwise reduced to a hexahydro-2-naphthoyl-CoA with unknown conformation of the remaining double bonds (Eberlein *et al.*, 2013). Furthermore, *cis*-2-carboxycyclohexylacetic acid could be identified as a metabolite of the down-stream pathway (Annweiler *et al.*, 2002).

### **Objectives**

Our aim for this study was the elucidation of the next metabolites of the pathway following *cis*-2-carboxycyclohexylacetyl-CoA and the identification of the enzyme reactions finally leading to the second ring-cleavage.

### **Methods**

The metabolite *cis*-2-carboxycyclohexylacetic acid was chemically synthesised and converted to the corresponding coenzyme A thioester. The latter was tested for conversion in cell-free-extracts of N47 and NaphS2 in the presence of different potential co-factors and electron acceptors. Samples were analysed by HPLC and emerging metabolites were further characterised via GC-MS.

### **Conclusions**

Conversion of *cis*-2-carboxycyclohexylacetyl-CoA was only observed in the presence of ferrocenium hexafluorophosphate, which can oxidise enzyme-bound co-factors like FADH<sub>2</sub>. The first emerging metabolite had a double-bond introduced, indicating the activity of a FAD-dependent acyl-CoA dehydrogenase. Also the product of the next enzyme, probably a hydratase, could be detected. Combining these data, we can for the first time make a detailed proposal for the steps leading to the second ring-cleavage in the down-stream pathway of anaerobic naphthalene degradation.

**FEMS-2517**  
**Anaerobic physiology**

**EXPLORING A RICE STRAW DEGRADING MICROBIAL COMMUNITY ENRICHED FROM SWINE MANURE DIGESTER MATERIAL**

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**Background**

Rice straw is one of the most abundant agricultural waste residues in the world. Currently it is burned in the fields, which causes pollution and greenhouse gas emission, whereas it has potential as feed for biogas production.

**Objectives**

To understand and stimulate biogas production from rice straw, we aim to isolate novel microbes with metabolic capacities that are key in the degradation of rice straw. Ingham et al. (2007) developed the MicroDish Culture Chip (MDCC) for cultivation of micro-colonies.

**Methods**

We set up a method for using the MDCC in anaerobic conditions which allows for screening key capacities and studying microbial interactions.

**Conclusions**

After five weeks incubation of anaerobic liquid cultures at 37°C, 75-80% of the rice straw dry weight was degraded and 250 L\*kg VS<sup>-1</sup> methane was formed, and acetate, propionate, benzoate and 3-phenyl propionate were measured as intermediates. The rice straw-degrading community was recently transferred to specific substrates. After 25 days of incubation methane was formed from cellulose and xylan, but not from lignin.

**Reference**

Ingham CJ, Sprengels A, Bomer J, Molenaar D, van den Berg A, van Hylckama Vlieg JE, de Vos WM; The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms; Proc Natl Acad Sci U S A. 2007 Nov 13;104(46):18217-22. Epub 2007 Nov 7.

## FEMS-2203

### Antibiotic resistance and environment

#### GENE THERAPY PLASMID pORF-hIL12 TRANSFORMATION FREQUENCY AND EFFICIENCY INTO SELECTED BACTERIAL STRAINS: A RISK ASSESSMENT OF AMPICILLIN RESISTANCE GENE TRANSFER

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#### Background

Plasmid gene delivery vectors offer several advantages over viral vectors, including safety, lower toxicity and easier preparation. Selective marker genes coding for antibiotic resistance have been traditionally an essential part of these plasmids. However, health authorities recommended the use of plasmids without resistance markers in order to prevent the spread of resistance genes.

#### Objectives

The aim of our study was to determine the possibility, frequency and efficiency of therapeutic plasmid pORF-hIL12 Amp<sup>R</sup> transformation into different bacterial strains.

#### Methods

The strains used were selected from 69 isolates belonging to 17 genera, which were isolated from dogs skin before application of electrogene therapy (EGT) (1) and from 89 isolates belonging to 17 genera retrieved after EGT. Several enterobacterial strains were also included into the study. Transformation of electrocompetent bacteria from genera *Staphylococcus*, *Bacillus*, *Acinetobacter*, *Kocuria*, *Micrococcus*, *Rhodococcus*, *Streptomyces*, *Mycobacterium* and *Arthrobacter* yielded no transformants with pORF-hIL12. The transformation frequency (efficiency) of *Escherichia coli*, *E. hermanii*, *Buttiauxella* sp., *Salmonella enterica*, *Proteus vulgaris*, *Erwinia chrysanthemii*, *Yersinia* sp., *Providencia* sp., *Enterobacter* sp., *Shigella boydii*, *Herbaspirillum frisingense* and *Sfingomonas* sp. ranged between 10<sup>-4</sup> and 10<sup>-10</sup> (10<sup>-1</sup> and 10<sup>-5</sup>).

#### Conclusions

Our study revealed that pORF-hIL12 could be introduced into several enterobacterial species, if they are present on the dogs skin and naturally competent. Thus a potential risk for the spread of the ampicillin resistance gene from pORF-hIL12 by horizontal gene transfer can not be excluded.

#### Reference

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**FEMS-2383**

**Antibiotic resistance and environment**

## **IN VITRO SUSCEPTIBILITY ACTIVITIES OF EIGHT ANTIFUNGAL DRUGS AGAINST CLINICAL AND ENVIRONMENTAL ISOLATES OF PHAEOACREMONIUM SPECIES**

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### **Background**

Phaeoacremonium species are chiefly found in the environment and can either cause a plant disease which is an uncommon cause of human disease. Most cases of Phaeoacremonium infection in humans involve traumatic inoculation and present as subcutaneous abscesses, cysts, or osteoarthritis. The spectrum of disease caused by Phaeoacremonium species especially *P. parasiticum* is variable and ranges from subcutaneous infections to eumycetoma, arthritis, osteomyelitis, endocarditis, fungemia and disseminated disease.

### **Objectives**

Despite worrying clinical pictures associated with *Phaeoacremonium*, there is little information regarding the antifungal susceptibility patterns against currently available antifungal agents.

### **Methods**

Thus, the *in vitro* activities of eight antifungal agents against clinical and environmental strains of *Phaeoacremonium* were obtained. The Clinical and Laboratory Standards Institute (CLSI) provides no specific guidelines for testing the *in vitro* antifungal susceptibility of this fungus. No other testing protocol has also been validated for testing the susceptibility of *Phaeoacremonium*.

### **Conclusions**

In the present study, the resulting MIC<sub>90</sub>s for all strains were as follows, in increasing order: POS (0.125 µg/ml; VOR (0.5 µg/ml); AmB (0.5 µg/ml); ISA (1 µg/ml); CAS (8 µg/ml); ANID (8 µg/ml); ITC (16 µg/ml); and FLU (64 µg/ml). The difference in the MIC<sub>90</sub>s between the two groups of isolates did not differ by more than one

dilution. Therefore, the present study based on *in vitro* activity showed that posaconazole and voriconazole might have a potent activity with a best choice of alternative to amphotericin B. In

**FEMS-1374**

**Antibiotic resistance and environment**

**IMPACT OF WASTEWATER DISCHARGES IN SOILS: DEFINITION OF LIMITS OF DETECTION AND QUANTIFICATION OF ANTIBIOTIC RESISTANCE GENES**

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**Background**

Wastewater can contribute to the spread of antibiotic resistant bacteria and resistance genes (ARB&G) in the environment. The discharge of treated wastewater in soils may lead to the accumulation of different contaminants, including ARB&G. However, due to technical limitations and low abundance of ARB&G in water, it is possible that the effects remain unnoticed before a long term exposure has occurred.

**Objectives**

To test this hypothesis, we designed an experiment to assess the limits of detection of ARG in soil.

**Methods**

An agricultural soil was soaked with a synthetic effluent containing two ARB to reach bacterial densities from  $10^2$  up to  $10^9$  CFU/g. ARB were *Escherichia coli* strain A2FCC14, which harbours the genes *bla*TEM, *bla*CTX-M-15, *bla*OXA-1, *aac*(6')-Ib-cr and integrase Intl-1, and *Enterococcus faecalis* H1EV10, with the *vanA* gene.

ARB&G detection limits were assessed based on culture-dependent and culture-independent methods (PCR and qPCR).

**Conclusions**

The limit of detection by conventional PCR was gene-dependent and corresponded to bacterial densities between  $10^4$  (in the case of *aac*(6')-Ib-cr) and  $10^7$  CFU/g soil (in the case of *bla*CTX-M-15 and *bla*OXA-1). Using qPCR, the quantification limit corresponded to bacterial densities of  $10^3$  CFU/g soil, although detection was possible for lower genes abundances. However, these values can be considered high for certain up to now uncommon ARG, which can spread in soils remaining unnoticed. The relatively high detection limits observed will be discussed, highlighting the meaning of this shortcoming for an adequate risk assessment of the implications of wastewater discharges in soil.

## **FEMS-3077**

### **Antibiotic resistance and environment**

#### **FIRST SURVEILLANCE DATA ON SELECTED ANTIBIOTIC RESISTANCE DETERMINANTS AND MOBILE GENETIC ELEMENTS FROM WWTP EFFLUENTS THROUGHOUT EUROPE**

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### **Background**

Evolution of bacterial antibiotic resistance represents one of the most threatening health care problems worldwide. This problem urgently requires a better understanding of important reservoirs and paths of dissemination, such as those represented by wastewater treatment and reuse.

### **Objectives**

While coordinated European Surveillance programs exist for clinically-associated antibiotic resistance, equivalent programs addressing the (aquatic) environments are missing. The aim of this study was to obtain a first insight into the abundance of antibiotic resistance genes in the treated effluent discharged by urban wastewater treatment plants (UWTP) from distinct geographic areas. This is the first pan-European synopsis on the levels of antibiotic resistance genes in treated wastewater.

## **Methods**

Within the 2014 WG5 Norman campaign we screened 20 (UWTP) in 13 different nations in Europe to screen for five environmentally and medically relevant antibiotic resistance and related genes (ARG, blaTEM; vanA; qnrS; sul1; ctx-m 32 and intl1). For three consecutive days, 24h-composite samples were collected and processed immediately for DNA extraction.

## **Conclusions**

All analysed ARG were detected in samples from all countries, with differences between different UWTP effluents, suggesting that the investigated determinants are adequate indicators. A north to south gradient within Europe could be observed, with the northern UWTPs showing the lower abundance of ARGs in the effluent than southern countries. This study sets the base for further studies investigating correlations between resistances and treatment processes, and linking environmental and clinical occurrence.

**FEMS-0776**

**Antibiotic resistance and environment**

**VIROME-ASSOCIATED ANTIBIOTIC RESISTANCE GENES IN FRESHWATER  
AQUACULTURE**

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**Background**

Due to their structural characteristics in extracellular phase, bacteriophages persist quite successfully in the environment and are resistant to natural or man generated stressors. Aquatic compartments may therefore play a significant role in driving antibiotic resistance genes (ARGs) transfer.

**Objectives**

The project is aimed to highlight the impact of viral populations in the spreading of ARGs in aquaculture.

**Methods**

Upstream and downstream water samples from an experimental salmonid aquaculture were treated with a tangential flow filtration system to separate and concentrate viral-like particles and microbial cells. Then viromes were investigated through shotgun NGS while DNA from microbial communities underwent NGS 16S *rRNA* gene profiling. Bioinformatic analysis was performed using QIIME and Meta-Vir tools, CARD, NCBI, and COG database. The AR colonies frequency was evaluated by standard plating.

## **Conclusions**

Upstream and downstream samples were characterized by limited changes in viral community composition at family level, while a large variation in bacterial population was observed. The relative abundance of AR drug classes did not significantly change whereas the taxonomic distribution of ARGs and bacterial metabolic genes in the viromes were remarkably different between upstream and downstream samples thereby reflecting the taxonomic composition and diversity of the bacterial communities. Interestingly, an inverse relationship between the relative abundance of genes belonging to a bacterial family in the virome and the same bacterial family abundance in the microbioma was observed. Furthermore, the frequency of AR resistant colonies showed an inverse relationship with respect to the abundance of specific virome-associated ARGs.

**FEMS-2257**

**Antibiotic resistance and environment**

**CHARACTERIZATION OF YBJG, A PYROPHOSPHATE PHOSPHATASE FROM *E. COLI* INVOLVED IN THE LIPID CARRIER UNDECAPRENYL PHOSPHATE METABOLISM**

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**Background**

Undecaprenyl phosphate (C<sub>55</sub>-P) is an essential lipid carrier involved in the biosynthesis of cell surface carbohydrate polymers such as the peptidoglycan. C<sub>55</sub>-P is the result of the dephosphorylation of the undecaprenyl pyrophosphate (C<sub>55</sub>-PP) by specific phosphatases. In *Escherichia coli* this dephosphorylation can be performed by four integral membrane proteins, BacA, and three members of the type 2 phosphatidic acid phosphatase family (PAP2), PgpB, YbjG, and LpxT.

**Objectives**

The aim of this project is to characterize YbjG and contributes to the understanding of the physiological role and mechanism of action of this enzyme in the C<sub>55</sub>-P metabolism. The C<sub>55</sub>-PP phosphatases could become an interesting target in the search for new molecules with antibacterial activity.

**Methods**

In parallel the stability of YbjG and its activity against C<sub>15</sub>-PP were assessed in 94 different detergents. Moreover the enzymatic activity of YbjG was studied: substrate specificity, optimum pH and temperature, effect of detergent concentration.

**Conclusions**

For the first time, YbjG has been purified and we show its ability to dephosphorylate C<sub>15</sub>-PP, DGPP and C<sub>55</sub>-PP *in vitro* with respectively decreasing efficiency. No activity has been detected on five other potential substrates (PPi, PA, C<sub>5</sub>-PP, G6P & PNPP).

The phosphatase activity on C<sub>15</sub>-PP is maximum at pH 6,5 and 25 °C. Moreover Cymal6, LMNG, & ωUDM are good detergent both for the stability and the C<sub>15</sub>-PP



phosphatase activity of YbjG, but approximately half of the 94 tested detergents show C<sub>15</sub>-PP phosphatase activity on the qualitative enzymatic test.

**FEMS-1753**

**Antibiotic resistance and environment**

**CONSTITUTIVE PRESENCE OF ANTIBIOTIC RESISTANCE GENES WITHIN THE BACTERIAL COMMUNITY OF A LARGE SUBALPINE LAKE**

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**Background**

The fate of antibiotic resistance genes (ARGs) in natural bacterial communities is of primary concern as prodromal of their potential spread within pathogenic bacteria. Although of diverse origin, the persistence of ARGs in aquatic environments is highly influenced by anthropic activities, allowing potential control actions in well-studied environments. However, knowledge of their abundance and dynamics in ecosystems is still scarce.

**Objectives**

We investigated the presence and the abundance of twelve ARGs in large, subalpine Lake Maggiore (Italy/Switzerland), using qPCR, at different stations, depths and seasons. In order to compare seasonal dynamics to short-term changes we sampled one station daily over the course of a week. We then evaluated correlations between ARGs and the bacterial community composition assessed with Illumina sequencing of the 16S rDNA and a number of environmental parameters, including bacterial density, temperature, chlorophyll, oxygen and water transparency.

**Methods**

**Conclusions**

Our results suggest the constitutive presence of at least three to four ARGs within the natural bacterial community, with a high proportion of bacteria potentially resistant to tetracyclines and sulphonamides. However, in some cases we also observed short-lived peaks of ARGs (one day) which also co-occurred with a significant change of the bacterial diversity in the system. These observations, on the one hand propose the lake as an ideal reservoir for the persistence of certain ARGs, and on the other hand it suffers from point pollution with ARGs. This highlights the need for an appropriate management of the antibiotic consumption in the catchment basin and of the potential sources of contamination.

## **FEMS-1732**

### **Antibiotic resistance and environment**

#### **ANTIBIOTIC RESISTANCE OF BACTERIAL STRAINS ISOLATED FROM THE AIR OF SORTING FACILITIES**

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#### **Background**

The spread of resistant strains of bacteria in the community is recognized as a recent trend, and this fact adds new risk factors usually not taken into consideration in particular for professional activities where the exposure to biological agents is larger than usual, as is the case of waste sorting facilities.

#### **Objectives**

The study focuses on the characterization of the antibiotic resistance profile of *Staphylococcus* spp. and presumptive *Enterobacteriaceae* strains isolated from the air of sorting facilities.

#### **Methods**

A total of 49 *Staphylococcus* spp. strains isolated from air samples were used for the detection of *mecA* and *pvl* genes by RT-PCR. Phenotypic resistance has been determined by disk diffusion and agar dilution methods. From an initial screening of 133 air samples using McConkey agar containing meropenem (2mg/L), 37 Gram (-) strains were phenotypically characterized. Strains resistant to meropenem were characterized in terms of resistance to other 15 antibiotics of 7 different classes using the disk diffusion method.

#### **Conclusions**

None of the *Staphylococcus* spp. strains was *pvl* (+). The presence of the gene *mecA* was observed in 8% of the *Staphylococcus* spp. strains tested and 25 of the strains were able to grow in the presence of oxacillin concentrations between 0,5 mg/L and 4 mg/L. The presumptive *Enterobacteriaceae* strains isolated in the initial screening showed high prevalence of resistance to at least one antibiotic (95%) and 43% of the strains were resistant to 6 different antibiotics. Meropenem resistance was confirmed in 51% of the strains.

**FEMS-1873**

**Antibiotic resistance and environment**

**EXTENDED SPECTRUM BETA-LACTAMASE PRODUCING  
ENTEROBACTERIACEAE IN RECREATIONAL RIVER WATERS OF THE NORTH  
OF PORTUGAL**

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**Background**

River water is one of the most important reservoir and vehicle of bacterial resistance dissemination, in nature. The overexposure of aquatic environments to human and animal intestinal commensals and pathogens, and the selective pressure exerted by factors as antibiotic residues, are important sources of resistance. Our previous work showed multi-resistant ESBL producing *E.coli*, in river water in Portugal, leading us to improve our research about this public health concern.

**Objectives**

Our aim was the detection of ESBL and carbapenemase producing Enterobacteriaceae in recreational river waters in the North of Portugal.

**Methods**

Water samples of 5 different rivers with recreational activities, were analysed by membrane filtration on MacConkey agar and MacConkey Agar with cefotaxime, ciprofloxacin and meropenem. Representatives of different colony morphotypes were tested for antimicrobial susceptibility and carbapenemase screening, according to CLSI and EUCAST. ESBL producers were confirmed by the double-disk-synergy-test and PCR was performed for detection of *bla*<sub>TEM</sub>, *bla*<sub>OXA</sub>, *bla*<sub>SHV</sub>, *bla*<sub>CTX-M-group-1</sub> and *aac(6')-Ib-cr* genes. Isolates were identified by ID-32-GN.

**Conclusions**

Our study showed the dissemination of *bla*<sub>CTX-M-group-1</sub> producing Enterobacteriaceae in this aquatic environment and the presence of Gram-negative-non-fermentative bacteria with reduced susceptibility to carbapenems. From 56 isolates analyzed, 33 showed multi-resistance, 17 were ESBL producers. Isolates of *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter* spp. showed positive for *bla*<sub>CTX-M-group-1</sub> (13), *bla*<sub>OXA</sub> (12), *bla*<sub>TEM</sub> (1), *bla*<sub>SHV</sub> (6) and *aac(6')-Ib-cr* (1), genes. Results indicate that recreational river waters of the North of Portugal are reservoirs of ESBL producing Enterobacteriaceae and non-fermentative-Gram-negatives with reduced susceptibility to carbapenems, suggesting a public health concern.

## **FEMS-1899**

### **Antibiotic resistance and environment**

#### **CATTLE AS RESERVOIR OF CIPROFLOXACIN RESISTANT COLIFORMS**

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#### **Background**

Human intestinal colonization by antimicrobial resistant *E.coli*, suggests environmental reservoirs as water, food or direct contact with animals. Descriptions of fecal carriage in food-producing animals, might suggest cross-transmission between humans and animals or a common environmental source. Meat can be a source of resistant bacteria that can spread through the food chain.

#### **Objectives**

The aim of our study was the detection of ciprofloxacin resistant (CR) coliforms in cattle fecal flora of two different type facilities in the North of Portugal.

#### **Methods**

Faecal samples were suspended in TSB and incubated at 37°C overnight. Isolates were selected on MacConkey agar with and without ciprofloxacin. Colonies of lactose fermenters were randomly selected and susceptibility to antimicrobial agents was determined by the agar diffusion test using CLSI guidelines. Strains were identified by ID 32 GN and API 20 E. Genetic information was investigated for *qnr* and *aac(6')-Ib-cr* genes, by PCR.

#### **Conclusions**

Our study showed the emergence of *qnrA* and *aac(6')Ib-cr* mediated ciprofloxacin resistance in coliforms from Portuguese cattle. CR *E.coli* isolates were detected in all samples of intensive production. ESBL producers were detected among the CR isolates, and resistance to tetracycline, chloramphenicol, trimethoprim-sulfamethoxazole,  $\beta$ -lactams and  $\beta$ -lactam/inhibitor association, was frequent.

Intestinal flora of cattle might act as reservoir of CR coliforms. Fluoroquinolones used in veterinary suggest a potential for selection of CR coliforms in the faecal flora of

production animals. Bovines can be a source of resistant bacteria colonization through the food chain, by unexpected contamination of carcasses by CR fecal isolates.

## **FEMS-2801**

### **Antibiotic resistance and environment**

#### **ACQUIRED PLASMIDS HAVE A ROLE IN THE FITNESS AND PHENOTIPIC CHARACTERISTICS OF BACTERIA**

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**Background** The spread of antibiotic resistance among bacteria has become one of the most important health problems nowadays. The problem relays on the dissemination of antibiotic resistance genes by conjugative plasmids. Increasing antibiotic concentrations in clinical and natural environments enhances this problem. Many antibacterial compounds have their roots in natural environments, such as soil. As a consequence, bacteria have coevolved and shared resistance mechanisms and genes along evolution.

**Objectives** This work is focused on the search of new conjugative plasmids from environmental unique soils in which high concentrations of antibiotics are present as a result of animal manure amendments. Additionally, we aim to study the effect of these plasmids on the fitness and phenotype of recipient bacteria.

**Methods** Exogenous Plasmid Isolation technique was used to collect conjugative plasmids from the bacterial community present in different soils. We obtained a total of 17 *E. coli* transconjugants from 10 different soils selected with ampicillin, erythromycin, chloramphenicol or streptomycin. To study the effect that the acquisition of these plasmids has on the fitness and phenotype of *E. coli* transconjugants, we determined growth curves, antibiotic MIC values, and community-level physiological profiles (BIOLOGTM) which provide information on carbon substrate utilization patterns.

**Conclusions** We conclude that acquired conjugative plasmids affect both fitness and phenotype of recipient bacteria.

## **FEMS-1370**

### **Antibiotic resistance and environment**

#### **EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI SEQUENCE TYPE 131 IN HEALTHY BROILER CHICKEN MEAT IN ITALY: A COMBINATION OF ANTIBIOTIC RESISTANCE WITH VIRULENCE**

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#### **Background**

Extraintestinal pathogenic *Escherichia coli* sequence type 131 (ExPEC-ST131) has emerged internationally as a multidrug-resistant pathogen. It has been shown that the transmission can occur from animals to humans. Based on existing evidence, poultry is the food animal source most closely linked to human *E. coli*.

#### **Objectives**

The objective was to characterize ExPEC from raw chicken meats purchased from Palermo, Italy.

#### **Methods**

In a total of 187 raw chicken meat from retail markets in Palermo, Italy during March-November 2014, selective cultures and disk-diffusion assays for the isolation and characterization of antimicrobial-resistant *E. coli* were performed. Polymerase chain reaction-based assays were done to detect Extended-Spectrum  $\beta$ -Lactamases genes (ESBLs), plasmid-mediated AmpC genes, plasmid-mediated quinolone resistance (PMQR) genes and *aac(6')-Ib* genes, phylogenetic group typing and to define ExPEC-associated traits. A single nucleotide polymorphism (SNP) PCR was done to detect *E. coli* ST131.

#### **Conclusions**

In total, 179 *E.coli* Isolates were isolated from 187 chicken meat samples. Fifteen (8.4%) isolates were belong to phylogenetic group B2, at least one of the ESBLs genes and/or PMQR determinants were detected in all of these isolates. Three isolates of group B2 contained *aac(6')-Ib-cr*. Based on the molecular definition of ExPEC, all of the B2 isolates had the status of ExEPC. The most frequent virulence factors were kpsMT II and iutA. SNP- PCR results showed that eight isolates were ST131 which is the first report from Italy and poses a potential zoonotic risk.



Investigations into the ability of our poultry ExPEC to cause human infections are warranted.

**FEMS-1391**

**Antibiotic resistance and environment**

## **PSEUDOMONAS AERUGINOSA GENE EXPRESSION PROFILING FOR ENVIRONMENTAL MONITORING**

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### **Background**

A major contributor to water pollution is the discharge of domestic and industrial waste into the environment. Although developed countries treat wastewater and sludge before releasing it into the environment, many contaminants are not completely removed by the wastewater treatment plants (WWTP) and subsequently end up in environmental waters. There are concerns that these contaminants contribute to increased virulence and/or antibiotic resistance in microbes in the environment and thus pose a health risk.

### **Objectives**

To design and apply a multi-gene expression system for *Pseudomonas aeruginosa* to predict the potential impact of soluble components released from sewage sludge on bacteria.

### **Methods**

Selected genes associated with stress response, virulence and antibiotic resistance were analysed by quantitative PCR in *P. aeruginosa* subjected to tobramycin or heat shock treatments for validation, or sewage sludge leachates from 3 Swedish WWTPs (Eskilstuna, Örebro and Västerås). *P. aeruginosa* was selected because it is normally in the environment and is also an opportunistic pathogen. The molecular profiles of the sludge leachate treatments were subjected to principle component analysis.

### **Conclusions**

The sludge leachate from Eskilstuna significantly affected more *P. aeruginosa* genes, responsible for general stress response and virulence, while sludge leachate from Västerås affected fewer genes and clustered more closely to the control. Örebro sludge leachate produced a different gene expression profile from the other two WWTPs. This suggests that the presented strategy of *P. aeruginosa* toxicogenomics has potential applications in evaluating the effects of soluble contaminants on bacteria in environmental waters for considerations in risk assessment.

**FEMS-0841**

**Antibiotic resistance and environment**

## **CARRIAGE OF CMY-96 MEDIATED CEPHALOSPORIN RESISTANCE IN ESCHERICHIA COLI OF FOOD ORIGIN: A REPORT FROM INDIA**

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### **Background**

**Background:** Expanded spectrum cephalosporin resistance continues to be a global threat with regard to infection control management and therapeutic options. *Escherichia coli*, being versatile in host tropism, acts as a major contributor in horizontal and vertical expansion of antibiotic resistance even without external antibiotic pressure. Food remains an important growth media for both pathogenic and non-pathogenic bacteria and a source for genetic exchange between them leading to transfer of antibiotic resistance. Thus, knowledge of these resistance determinants in non-clinical host is critical in tracking down their dissemination in trans specific level.

### **Objectives**

**Objectives:** The current investigation was undertaken to assess the occurrence of class C beta lactamase mediated cephalosporin resistance and their transmission dynamics among non-clinical isolates of *E. coli* in a high altitude city of Northeastern India.

### **Methods**

**Methods:** A total of 56 *E. coli* were recovered from 61 ready to eat food samples. Phenotypic and molecular characterization of AmpC genes was performed and MIC against oxy-iminocephalosporins was also determined. Isolates positive for *bla*<sub>CIT</sub> was checked with CMY specific primers and amplicons were sequenced. Horizontal transferability was tested by conjugation and transformation. Plasmids were further characterized by PCR based replicon typing.

### **Conclusions**

**Conclusion:** A total of 17 isolates were found harbouring *bla*<sub>CMY-96</sub> type. The gene was conjugatively transferable and maintained within W inc type plasmid of approximately 23 kb in size. Carriage of this rare type resistant determinant is of clinical and epidemiological interest as non-pathogenic organism may act as vehicle for their dissemination in community and within hospital environment.



**FEMS-2789**

**Antibiotic resistance and environment**

## **ANTIFUNGAL SUSCEPTIBILITY OF ASPERGILLUS FLAVUS RECOVERED FROM CLINICAL AND ENVIRONMENTAL SPECIMENS IN IRAN.**

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### **Background**

*Aspergillus flavus* is the second leading cause of invasive and non-invasive aspergillosis, also common cause of fungal sinus and cutaneous infections. Since resistance to these drugs has been seen in patients, susceptibility testing can help in defining the activity spectrum of an antifungal and determining the appropriate drug for treatment.

### **Objectives**

The aim of this study was to analyse the in vitro activities of amphotericin B, itraconazole, voriconazole, posaconazole, and caspofungin against clinical isolates of *A. flavus* recovered from clinical and environmental specimens in Iran.

### **Methods**

199 isolates of *A. flavus* (171 clinical and 28 environmental) were included in the study. All isolates were identified by typical colony and microscopic characteristics, also characterized by molecular methods. MICs and MECs were determined according to recommendations stated in the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document

### **Conclusions**

The range of MIC/MEC and MIC/MEC<sub>90</sub> values were as follows: amphotericin B, 0.031-8 and 1 µg/ml; voriconazole, 0.031-8 and 1 µg/ml; itraconazole, 0.031-2 and 1 µg/ml; posaconazole, 0.008-0.5 and 0.341 µg/ml; and caspofungin, 0.008-0.25 and 0.063 µg/ml, respectively. Caspofungin was the most active drug, followed by posaconazole, itraconazole, Voriconazole, and amphotericin B. Caspofungin exhibited highly potent activity (100% of strains had MEC<sub>50</sub> and MEC<sub>90</sub> values of ≥0.5 µg/ml) against *A. flavus* strains. Among the azoles, posaconazole (MICs ≥1 µg/ml) was the most potent followed by itraconazole and voriconazole. We were not able to find significant differences in antifungal susceptibilities between strains recovered from clinical and environmental specimens (P >0.01).



## **FEMS-1600**

### **Antibiotic resistance and environment**

#### **HEAVY METAL TOLERANCE OF STAPHYLOCOCCUS AUREUS ISOLATES FROM PATIENTS TREATED WITH SILVER COATED WOUND DRESSINGS**

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#### **Background**

Silver coated wound dressings are a new useful tool to fight bacterial infections. In vitro studies with heavy metal surfaces showed good results but only by direct contact with the patch and with a dry metal surface. But the efficacy of the coatings seems to be less effective over a longer application time.

#### **Objectives**

The aim of the study was to test heavy metal tolerance of *Staphylococcus aureus* wound isolates that had contact to silver coated wound dressing in comparison to isolates that had not.

#### **Methods**

120 *Staphylococcus aureus* patients isolates (MRSA and MSSA, with or without metal contact). Strains were analysed in a Bioscreen device over a period of 72 hours, with copper concentrations up to 20 mM copper chloride. Additionally the strains were screened for genes thought to be responsible for heavy metal tolerance (*copA*, *copB*).

#### **Conclusions**

Some isolates of both groups showed high tolerance levels for copper chloride. Surprisingly MRSA (28% positive for *copB*) came up with an overall lower metal tolerance (around 8mM) in contrast to MSSA with 9mM. Prior contact to silver dressing did not show any difference in their metal tolerance. MRSA were positive in 28% for *copB* whereas MSSA were positive in 10%. Interestingly some of the high tolerance strains showed a prolonged lag-phase over days. Therapy with silver dressings does not favor strains with higher metal tolerance. In contrast to other studies the Austrian MRSA showed lower copper tolerance than MSSA





**FEMS-2410**

**Antibiotic resistance and environment**

**CHARACTERIZATION OF LIPID COMPOSITION OF VANCOMYCIN-RESISTANT STAPHYLOCOCCUS EPIDERMIDIS 33 GISK**

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**Background**

Numerous studies have revealed that the emergence of vancomycin-resistant staphylococcal phenotype is a result of multiple mutations that markedly change biological properties of these bacteria.

**Objectives**

The goal of these investigations was the analysis of adaptation changes of *S. epidermidis* 33 GISK (Moscow) lipid composition and *S. epidermidis* 33 GISK Van<sup>r</sup> (its derivative strain) obtained via selection on media with increasing vancomycin concentrations.

**Methods**

Extraction of bacterial lipids was realized following the modified Bligh and Dyer method. Silica-gel plates Sorbfil (Russia) were used in thin-layer chromatography of lipid extracts in chloroform-methanol-water system (65:25:4, v/v). Identification of individual lipids was implemented via their functional groups' staining with specific dyes. Analysis of fatty acid methyl esters was made using RTX-5MS column (Restek) on gas chromatograph 6890N with MS-detector 5973N (Agilent Technologies). Chromatography was carried out in autonomous temperature mode (180 to 280°C) at a rate of 5°/min. Peak substances were identified with NIST98 database software library.

**Conclusions**

Increase of bacterial resistance to vancomycin is accompanied by augmentation of cardiolipin and phosphatidylglycerol content in cell membrane lipid components, and the occurrence of lysyl phosphatidylglycerol and phospholipid lysoforms therein, but decline in glycolipid content.

According to data obtained the main point of lipid composition alteration in Van<sup>r</sup> strain was the occurrence of unidentified phospholipid containing the alanine residue. Gas-chromatographic assay demonstrated that saturated and mono-unsaturated fatty acids C<sub>16</sub> and C<sub>18:1</sub> were the predominant components of Van<sup>r</sup>-lipids, whereas fatty acids C<sub>15</sub> and C<sub>17</sub> were typical for the initial strain.

**The work was supported by RFBR (14-04-00687\_a).**



## **FEMS-2737**

### **Antibiotic resistance and environment**

#### **GENETIC DIVERSITY AND ANTIBIOTIC RESISTANCE OF CLINICAL AND NON-CLINICAL ISOLATES OF ESCHERICHIA COLI (E. COLI) IN GRENADA**

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#### **Background**

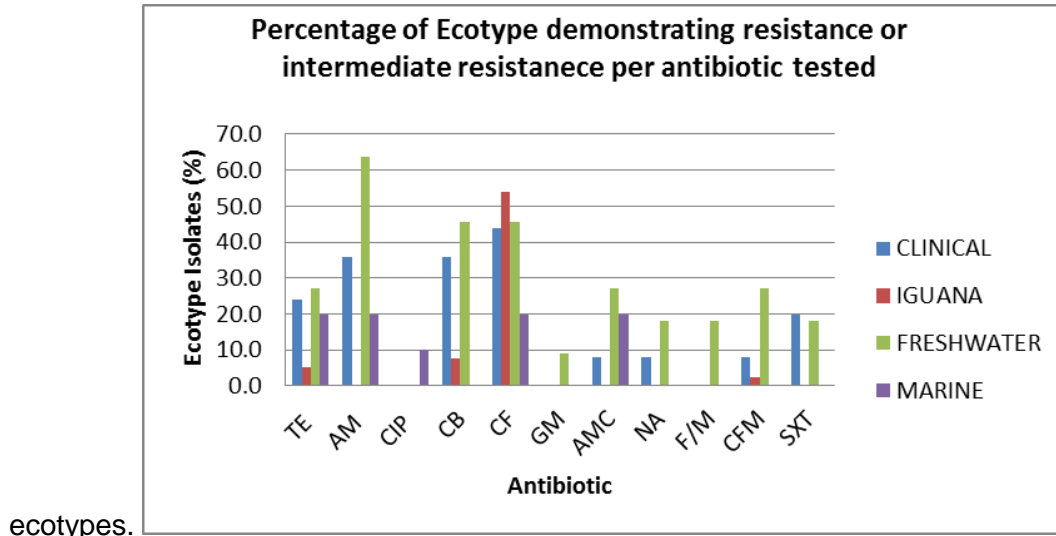
*E. coli* exhibits an increasing resistance to broad spectrum antimicrobial agents as well as the subsequent generations of these drugs. Previous genetic diversity studies indicated transmission of food-borne *E.coli* strains to human patients. Therefore it is important to study the genetic diversity and antibiotic-resistance patterns both in clinical and environmental *E. coli* to predict potential of transmission of organisms and genes antibiotic resistance.

#### **Objectives**

The aim of this study was to compare the genetic diversity and the antibiotic resistance patterns of uropathogenic *E.coli* (UPEC), to strains from freshwater, seawater and iguanas.

#### **Methods**

*E.coli* isolates were identified using API20E and ATCC 25922, Rep-(GTG)<sub>5</sub> PCR and BOX-PCR extragenic DNA fingerprinting, and FigTree™. The antibiotic resistance was assessed using the Kirby-Bauer disc diffusion against the eleven most commonly prescribed antibiotics in Grenada. Excel t-test and Statistica™ were utilized for comparison of patterns of drug resistance among four



## Conclusions

Both DNA fingerprinting methods targeted non-protein coding or extragenic DNA and demonstrated enormous diversity within the population of the studied bacteria. UPEC expressed resistance patterns similar to the marine for 50%, while both to iguana and to fresh water isolates for 72.7% of drugs tested. There were only three drugs which showed significant difference between the UPEC and the freshwater isolates, namely ampicillin, amoxicillin/clavulanate and nitrofurantoin. We identified resistance to  $\beta$ -lactam antibiotics and lack of resistance to ciprofloxacin and gentamycin both in clinical strains of *E.coli*. The fractions of drug resistance strains and resistance patterns of the UPECs were similar to *E.coli* isolated from natural sources in Grenada.

**FEMS-0750**

**Antibiotic resistance and environment**

**MOLECULAR EPIDEMIOLOGY OF CARBAPENEM RESISTANT  
ACINETOBACTER BAUMANNII (CRAB) IN SEPTICAEMIC & MENINGITIS  
PATIENTS IN A TERTIARY CARE HOSPITAL**

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**Background**

Due to escalating resistance to a range of antimicrobial agents, carbapenem resistant *Acinetobacter baumannii* (CRAB) has become a worldwide concern. Rapidly increasing prevalence of CRAB in many parts of the world in the past few years has questioned the reliability of the carbapenems.

**Objectives**

This study aimed to correctly identify *A. baumannii* species, antimicrobial susceptibility, and clonal relationships of CRAB clinical isolates.

**Methods**

Clinical isolates of *A. baumannii* collected from Clinical Bacteriology Laboratory between Sept 2013 and June 2014 were used in this study. Genospecies identification was verified by MALDI-TOF, and using polymerase chain reaction for *OXA-51* & *gyrB* genes. MIC for meropenem, imipenem & doripenem was determined using agar dilution method. Antibiotic susceptibility was performed using Kirby-Bauer disc diffusion method. Multi-locus sequence typing (MLST) was performed to study clonal relationships.

**Conclusions**

Our results demonstrate clonal spread of CRAB in our hospitals. Further MLST studies on a nationwide basis would provide a better insight into the transmission and dissemination of CRAB clones.

**FEMS-0736**

**Antibiotic resistance and environment**

**IDENTIFICATION OF A HEAT STABLE BETA-LACTAMASE, WHICH IS PRODUCE BY BACILLUS CEREUS NBRC13494, INACTIVATES THE MEROPENEM**

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**Background**

Antibiotics are used for the medical care, the domestic animals growth, and the control of health administration. Some antibiotics exposed in the environment are stable and keep the activities, which potentially causes the potential ecological and human health issue. The penem antibiotics, which are hardly to be degraded, are frequently used in Japan.

**Objectives**

In order to reduce the antibiotics pollution, we aimed to isolate soil bacteria that produce meropenem digestion enzymes.

**Methods**

Genome sequence analysis reveals that the some *Bacillus* species potentially produce antibiotic degradation enzymes. We screened *Bacillus* strains that can digest the meropenem from Biological Resource center in Japan and the soils.

**Conclusions**

We found *B. cereus* strain NBRC13494 and three natural isolates, SUBC1002, SUBC1008, and SUBC1010. The MICs of meropenem were 6.7, 2.5, 3.3, 3.4 mg/l, respectively. *B. cereus* strain NBRC13494 possessed the highest beta-lactamase activity among them. We found that the supernatant of *B. cereus* NBRC13494 after the heat treatment at 80 °C was also effective to degrade the meropenem. We resolved the proteins included in the supernatant of *B. cereus* NBRC13494 by SDS-PAGE and found major two bands with the molecular mass of 28-kDa and 33-kDa bands. We determined their amino acid sequences by LC-MS/MS. The 28-kDa protein corresponded to the *B. cereus* 5/B/6 beta-lactamase II. The 33-kDa protein corresponded to the *B. cereus* 5/B beta-lactamase I. These beta-lactamases belong to the different protein family and highly conserved among *B. cereus* group. These results suggested that some beta-lactamases produced by *B. cereus* strains potentially digested the meropenem.

## **FEMS-0723**

### **Antibiotic resistance and environment**

#### **IDENTIFICATION AND PREVALENCE OF ANTIBIOTIC RESISTANCE GENES IN FRESHWATER URBAN WATERSHEDS**

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#### **Background**

Antibiotic resistance genes (ARGs) of environmental origin have been identified as a potential source of the rise in antibiotic resistance in communities. To understand the risk of ARGs in source waters, identification and quantification of ARGs in watersheds is necessary. Here, two urban watersheds with different land-use profiles were studied: a river situated in an urbanized environment and a lake situated in a reserved environment.

#### **Objectives**

To identify and quantify ARGs using GeoChip microarray and quantitative PCR (qPCR).

#### **Methods**

To screen for ARGs, GeoChip version 5, which target 17 families of ARGs, was used to screen DNA extracted from three sampling sites at each watershed. Primers for qPCR were designed based on ARGs yielding the highest GeoChip intensities.

#### **Conclusions**

Based on GeoChip analysis, ABC transporters, multidrug and toxic efflux, multidrug resistance efflux pumps, multi-facilitator superfamily (MFS), small multidrug resistance (SMR),  $\beta$ -lactamases (A-D), tetracycline resistance (*tet*), vancomycin, virginiamycin resistance gene B (*vgb*), fosfomycin resistance (*fosA*, *fosB*, *fosX*) and quinolone resistance (*qnrC*) were detected in both watersheds. There was significantly higher intensity of  $\beta$ -lactamase A ( $p<0.05$ ), MFS ( $p<0.01$ ),  $\beta$ -lactamase C ( $p<0.01$ ), SMR ( $p<0.01$ ), and *vgb* genes ( $p<0.01$ ) in the urban environment than in the reserve; contrarily, *fosA* had a higher intensity in the reserve.

Quantification of *tet* and MFS revealed presence of these genes in low relative abundance (<2.5%) to 16S rRNA genes in both watersheds. Taken together this data

suggests that there are more ARGs in the urban than the reserve watershed and that only a sub-population of the bacterial community harbours these genes.



**FEMS-2458**

**Antibiotic resistance and environment**

**CARRIAGE TRANSMISSION AND SELECTION OF ANTIBIOTIC-RESISTANT  
ESCHERICHIA COLI IN AN EXPERIMENTAL BIRD MODEL**

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**Background**

Very low concentrations of antibiotics, more than a hundred times lower than the minimal inhibitory concentration (sub MIC), can select for antibiotic resistant bacteria *in vitro*.

**Objectives**

To investigate if sub MIC concentrations of antibiotics in the environment can select for antibiotic resistant bacteria present in the intestine of mallards and to study if and how rapidly different ESBL *E.coli* strains transmit within a flock of mallards.

**Methods**

In the selection study birds were infected with an equal amount of two isogenic ESBL producing *E. coli* strains where one is resistant to ciprofloxacin. Mallards were exposed to different concentrations of ciprofloxacin in the drinking water. Faecal samples were analysed and the ratios of ciprofloxacin resistant over ciprofloxacin susceptible bacteria were calculated.

In the carriage and transmission study four different ESBL *E. coli* strains with different resistance patterns were used to inoculate a group of mallards. Faeces samples from each bird were collected up to 29 days post infection and colonies were patched onto selective plates to follow each of the inoculated strains.

**Conclusions**

Low levels of antibiotics (<20 ng/ml) in the water source of birds enrich for antibiotic resistant bacteria. Resistant bacteria can be carried in a flock of birds for at least a month without any antibiotic selective pressure and antibiotic resistant bacteria rapidly spread in the flock of birds.

**FEMS-1423**

**Antibiotic resistance and environment**

**EXTRACELLULAR VESICLES AND RESISTANCE OF MYCOPLASMAS TO FLUOROQUINOLONES: RESISTOME OF *ACHOLEPLASMA LAIDLAWII***

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**Background**

Control of mycoplasma infections presents a serious problem which solution is connected with elucidating molecular-genetic mechanisms of mycoplasma adaptation to environments. Sequencing of bacterial genomes provides an opportunity for application of postgenomic technologies to study the above-mentioned processes. In our studies it was shown for the first time that adaptation to environments of *Acholeplasma laidlawii* – the 'ubiquitous' mycoplasma which is the major contaminant of cell cultures and the causative agent of phytomycoplasmoses, was associated with not only specific modulation of transcriptome and proteome profiles of the bacterium but secretion of extracellular vesicles.

**Objectives**

Comparative analysis of genome and proteome profiles and vesicular content of *Acholeplasma laidlawii* strains with different susceptibility to ciprofloxacin was the object of our work.

**Methods**

The TEM, SEM, LC-ESI-MS/MS and DNA sequencing were used in our study

**Conclusions**

As a result of our studies it was found that *A. laidlawii* strains with different susceptibility to ciprofloxacin differed significantly on complete nucleotide sequences of genomes, vesiculation level, cellular and vesicular proteomes. Furthermore, it was revealed that the extracellular vesicles of *A. laidlawii* mediated export of ciprofloxacin as well as mutant nucleotide sequences of the genes that encoded the antibiotic-targeted proteins. The obtained data provided a necessity for correction of current notion about the mechanisms that facilitate the rapid development of resistance to antibacterial agents in mycoplasmas and approaches to control of mycoplasma infections.

This work was supported by the grant RFBR 14-04-00883 A, NSH-1890.2014.4.

## FEMS-2062

### Antibiotic resistance and environment

#### DOES THE HOST MAKE A DIFFERENCE IN THE SPREAD OF ANTIBIOTIC RESISTANCE GENES IN WATER?

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#### Background

Hospital and municipal effluents receive high doses of antibiotic resistant bacteria and their genes (ARB&ARG), some of which can persist in the environment. Little is known about the factors influencing the fate of ARG when they are introduced in complex microbial communities.

#### Objectives

This study aimed at assessing the fate of some indicator genes hosted by different bacterial species when inoculated in wastewater.

#### Methods

Assays were performed in microcosms of hospital effluent, raw wastewater, treated wastewater and sterile ultrapure water. Replicas of these microcosms were inoculated with *Acinetobacter johnsonii* (*intl1*), *Escherichia coli* (*bla*<sub>TEM</sub>, *intl1*) and *Enterococcus faecium* (*vanA*) or with *Acinetobacter johnsonii* (*intl1*), *Klebsiella pneumoniae* (*bla*<sub>TEM</sub>, *intl1*) and *Enterococcus faecalis* (*vanA*). Inoculated and non-inoculated microcosms incubated for 15 days at 18 or 30°C, the genes were quantified by qPCR and bacterial community composition was assessed based on 16S rRNA gene-denaturing gradient gel electrophoresis.

#### Conclusions

In general, the bacterial community richness, diversity and evenness indices did not vary over the incubation period and similar results were observed at 18°C or 30 °C. Both *vanA* and *bla*<sub>TEM</sub> were observed to decay over time, with higher reduction than the genes 16S rRNA and *intl1*. The gene *vanA* decreased sharply after the incubation period irrespective of the gene host or type of water used in the microcosm. In contrast, the decay of the gene *bla*<sub>TEM</sub> varied with the host and type of water. Comparatively, *K.pneumoniae* presented a higher fitness in water than *E.coli*, suggesting the importance of the host in the spread of some ARG.

## **FEMS-2065**

### **Antibiotic resistance and environment**

#### **DIVERSITY AND ANTIBIOTIC RESISTANCE PATTERNS OF BACTERIAL ISOLATES FROM TWO RIVERS IN NORTHERN PORTUGAL**

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Portugal

<sup>2</sup>Águas do Douro e Paiva, S.A., Porto, Portugal

#### **Background**

Aquatic environments are important habitats for bacteria and water is a privileged mode of dissemination of microorganisms. Freshwater is inhabited by different bacterial populations, mostly strictly environmental, in pristine environments, and also of human/animal origin, in human-impacted areas. The rivers that flow in urban areas are often inhabited by both environmental and animal/human derived microorganisms.

#### **Objectives**

The study of the diversity and antibiotic resistance patterns of bacteria colonizing freshwater habitats brings interesting insights into the role of the natural and human-derived microbiota in the evolution of antibiotic resistance. This study explored the taxonomic diversity and antibiotic resistance patterns of bacteria isolated from freshwater.

#### **Methods**

Water and/or sediment samples were collected from rivers Ferreira (43 km) and Douro (897 km), in Northern Portugal. Heterotrophic bacteria were enumerated and isolated on plate count agar (PCA) or on PCA supplemented with antibiotics (PCA+A). The isolates (n=173) were characterized for their antibiotic resistance phenotypes and multidrug resistant (MDR) bacteria (to antibiotics of  $\geq 4$  classes) were identified based on 16S rRNA gene sequence analysis.

#### **Conclusions**

In both rivers, PCA counts ranged  $10^5$  CFU/g of the sediments being about 1-3 log less on PCA+A (ciprofloxacin, meropenem, gentamycin). In both Ferreira and Douro, the most prevalent antibiotic resistance phenotypes were for beta-lactams and sulfonamides and the least prevalent was for tetracycline. Ceftazidime, meropenem and ciprofloxacin resistance was more prevalent in Douro than in Ferreira isolates. About 40% of the isolates from PCA+A were MDR and the most common genera were *Pseudomonas*, *Chryseobacterium* and *Stenotrophomonas*, in Ferreira and *Chitinophaga* in Douro.

**FEMS-1769**

**Antibiotic resistance and environment**

**INDUCTION OF THE SOS RESPONSE BY ANTIBIOTICS THAT DO NOT TARGET DNA IN *V. CHOLERA***

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**Background**

The bacterial SOS response is a global stress response involved in DNA-damage repair. In *Escherichia coli* it is induced after DNA damage, for example after treatment with antibiotics that target DNA. The SOS response is also involved in genome plasticity and in the acquisition of resistance to antibiotics. Unexpectedly, the SOS response is induced in *Vibrio cholera* by exposure to sub-inhibitory concentrations (sub-MIC) of different families of antibiotics that do not target DNA, such as aminoglycosides, chloramphenicol and tetracycline. The use of a genetic screen led to the isolation of mutants in which induction of the SOS response by sub-MICs of aminoglycosides is lost. One of these mutants is inactivated for the *vc1636* gene, which encodes a putative DNA/RNA helicase.

**Objectives**

The purpose of this project is to characterize the protein encoded by the *V. cholerae* *vc1636* gene and to understand the induction of the SOS response by antibiotics that do not target DNA in *V. cholerae*.

**Methods**

Towards this goal we use several bacterial genetics assays.

**Conclusions**

The first results suggest that VC1636 plays an important role in DNA-damage repair.

**FEMS-2566**

**Antibiotic resistance and environment**

**ANTIMICROBIAL AND VIRULENCE FACTORS OF *V. PARAHAEMOLYTICUS* ISOLATED FROM THE FINAL EFFLUENTS OF WASTEWATER TREATMENT PLANTS**

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**Background**

Enteric pathogens, including *Vibrio* species, increasingly survive wastewater treatment processes, thus constituting threat to humans as final effluents are discharged into receiving water bodies used primarily as source of domestic or recreational waters. *V. cholerae* has been reported from various water sources however, other pathogenic species have been under reported in wastewater hence the focus is on *V. parahaemolyticus* for this study.

**Objectives**

The objectives of the study were to: Isolate and confirm presumptive *Vibrio* species while determining their incidence; Confirm *V. parahaemolyticus* using PCR; Determine the antibiogram characteristics of *V. parahaemolyticus* and determine the virulence factors specifically the *tlh* and *trh* gene of *V. parahaemolyticus*.

**Methods**

Wastewater final effluents were collected once, monthly, for a period of twelve months, membrane filtered and analysed in accordance with standard technique. Presumptive *Vibrio* isolates were confirmed via 16SrRNA gene and *V. parahaemolyticus* identified with *toxR* gene. Antimicrobial susceptibility test was done using 10 different antibiotics and virulence factors namely the thermostable direct haemolysin related haemolysins (*trh* gene) and thermolabile haemolysin (*tlh* gene) were assessed using PCR

**Conclusions**

Incidence of *Vibrio* species varied with months and treatment plants. Resistance was shown against certain antibiotics while in other antibiotics resistance varied. The incidence of *trh* genes was nil, while the *tlh* gene was 5.7 %. Although, the incidence of virulence determinants in *V. parahaemolyticus* were few, their presence portends a public health risk to the receiving water bodies used by humans and animals especially with the increasing resistance of the pathogens to antibiotics.

**FEMS-0514**

**Antibiotic resistance and environment**

**PHENOTYPIC AND MOLECULAR CHARACTERIZATION OF KLEBSIELLA SP FROM URINE SAMPLES OF IN- AND OUT-PATIENTS IN SOME HEALTH INSTITUTIONS IN NIGERIA**

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**Background**

*Klebsiella* species are important pathogens in nosocomial infections, most especially the multiple antibiotic resistant strains.

**Objectives**

The aim of this study was to isolate and identify *Klebsiella* sp from urine samples of in- and out-patients in some health institutions in Ile-Ife, Nigeria; determine the resistance pattern; detect plasmid DNA, resistance and virulence genes in multiple antibiotic resistant *Klebsiella* isolates.

**Methods**

Isolation of *klebsiella* sp was carried out on MacConkey agar plate at 37°C. Identification was done using standard techniques. Antibiotic sensitivity was determined using Kirby-Bauer's disc diffusion method. Plasmid DNA was extracted by alkaline lysis, resistance (*bla*CTX, *tet*M) and virulence (*cps* and *fim*H) genes were detected by PCR.

**Conclusions**

A total of 32 (7.02%) *Klebsiella* sp were identified, out of which 29 (90.63%) were multiple antibiotic resistant (MAR). Resistance to antibiotics varied greatly. The isolates showed high resistance to augmentin (93.75%) and tetracycline (71.88%). There was diversity in the MAR pattern with 21 different antibiotypes which ranged from two to ten antibiotics. Thirteen out of the 18 randomly selected MAR isolates harboured plasmids ranging from 900bp to 9416bp. Three (16.67%) of the isolates had *bla*CTX (543 bp) and *cps* (418 bp) genes while one (5.56%) harboured *fim*H (1250 bp) gene. However, *tet*M (974 bp) gene was not detected in any of the isolates. The incidence of MAR *Klebsiella* sp in the study is high and calls for great concern in health care delivery with great economic and health consequences.

## **FEMS-0800**

### **Antibiotic resistance and environment**

#### **AEROMONAS SPP. – B-LACTAMASES RESISTANCE IN WASTEWATER TREATMENT PLANT STRAINS**

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#### **Background**

Bacteria of the *Aeromonas* genus are fish and human pathogens commonly occurring in various aquatic ecosystems (Janda and Abbott, 2010). Literature data show a wide spread of drug resistance genes among these microorganisms with a high proportion of chromosomal and plasmid-mediated genes (Piotrowska and Popowska, 2014).

#### **Objectives**

The aim of this project was determination of antibiotic resistance genes in the  $\beta$ -lactam unsusceptible *Aeromonas* strains isolated from wastewater with different levels of purification. In addition, in drug-resistant strains, their plasmid profile and the conjugation potential was defined.

#### **Methods**

The double-disk synergy test was performed to determine the mechanisms of  $\beta$ -lactamases resistance (AmpC, ESBL, carbapenemases). In addition, disk-diffusion were performed to determine the bacterial antibiogram. Identification of resistance genes was performed by PCR (Multiplex) and confirmed by sequencing. Plasmids were isolated by alkaline lysis and commercial kits. Diversity of plasmids were evaluated by plasmid restriction profiles by comparison of their patterns obtained after digestion with different restriction enzymes. The conjugative transfer ability and host range of selected plasmids were performed according to standard methods.

#### **Conclusions**

*Aeromonas* strains existing in wastewater are  $\beta$ -lactam resistant and possess a variety of  $\beta$ -lactamases. We found and confirmed the genes encoding  $\beta$ -lactamase from variety of families e.g. OXA, TEM, SHV, GES, VEB, FOX and characteristic to *Aeromonas* spp. chromosomal metallo- $\beta$ -lactamase CphA. Some of the resistant strains possess plasmids which were characterized by a different profiles.



**FEMS-3038**

**Antibiotic resistance and environment**

**IMPACT OF URBAN CONTAMINATION OF LA PAZ RIVER ON THE ABUNDANCE OF FECAL COLIFORMS, AND ANTIBIOTIC RESISTANT GRAM-NEGATIVE BACTERIA IN RIVER WATER, SOIL AND VEGETABLES**

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**Background**

Worldwide, contamination of fresh water resources affects food security and ecosystem sustainability. This situation is aggravated in developing countries and markedly in dry regions such as the Bolivian Highlands. In this region along with a shortage of water, urban wastewater is directly discharged into fresh water bodies contributing to aquatic ecosystem degradation, as observed in La Paz River, which receives urban discharges that further contaminate agricultural lowlands, where vegetables and soil are directly irrigated by river water

**Objectives**

To evaluate the microbial quality of river surface water at unimpacted and impacted sites, and tested for antibiotic resistance of gram-negative bacteria isolated from river water, soil and vegetable samples.

**Methods**

Culture dependent methods.

**Conclusions**

High levels of fecal coliform density were found along impacted sites and green-leafy vegetable samples, exceeding at least in two and one order of magnitude respectively, the allowable concentration. Antibiotic resistance among gram-negative bacterial isolates was common at all sites. A spatial distribution of antibiotic resistance was found, where resistance frequency to not commonly used antibiotics was higher at unimpacted in contrast to impacted sites and vice versa, this distribution was associated with the density of fecal coliforms. Multiple Antibiotic Resistance (MAR) index values ranged from 0.176 to 0.310, being higher in soil samples. Overall, this study suggest that surface water, soil and vegetables can be reservoirs of antibiotic resistant gram-negative bacteria, highlighting health risk associated with vegetables production and consumption in the region.

**FEMS-2746**

**Antibiotic resistance and environment**

**QUINOLONE RESISTANCE IN LACTOBACILLUS  
CASEI/PARACASEI/RHAMNOSUS**

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**Background**

Resistance to quinolones has been little studied in lactobacilli and the breakpoint value established to discriminate resistant from sensitive strains, the same for all *Lactobacillus* species, has not been updated since 2003 (1).

High level of resistance is, in most cases, due to mutations in the quinolone resistance determining regions (QRDR) of the genes encoding DNA gyrase (*gyrA*) or topoisomerase IV (*parC*). Reduced sensitivity to quinolones is conferred by genes, among which the most widespread are the *qnr* genes for pentapeptide repeat family proteins that protect gyrase from quinolone inhibition, encoded by plasmids or integrons in Gram-negative bacteria and chromosomally in some Gram-positive bacteria (2).

**Objectives**

This study was aimed at understanding if the species of probiotic interest *Lactobacillus casei/paracasei/rhamnosus* may have a role in the spread of quinolone resistance.

**Methods**

Strains resistant to ciprofloxacin were selected among 184 *L. casei/paracasei/rhamnosus* bacteria by using the breakpoint concentration for lactobacilli (4 mg/l) in LSM medium. These were tested for the presence of mutations in the *gyrA* and *parC* QRDRs and of genes conferring quinolone resistance.

**Conclusions**

Among the 31 strains resistant to the breakpoint value for ciprofloxacin only one *L. rhamnosus* strain presented a mutation in the *gyrA* QRDR, not corresponding to those commonly associated to resistance. Results showed that quinolone resistance is intrinsic in some strains of *L. casei/paracasei/rhamnosus*.

**References:** 1. European Commission - Health & consumer protection directorate-general 2003. [ec.europa.eu/food/fs/sc/scan/out108\\_en.pdf](http://ec.europa.eu/food/fs/sc/scan/out108_en.pdf); 2. Ruiz et al. International Journal of Antimicrobial Agents 40 (2012) 196–203.



## FEMS-1349

### Antibiotic resistance and environment

#### SCREENING BACTERIA ISOLATED FROM AQUATIC ENVIRONMENTS FOR THE POTENTIAL TO DEGRADE SULFAMETOXAZOLE

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#### Background

In contrast to beta-lactam antibiotics, the biodegradation of sulfonamides seems to be uncommon in nature, in spite of the extensive use and environmental contamination associated with these drugs.

#### Objectives

The aim of this study was to investigate the potential of sulfonamide resistant bacteria isolated from wastewater, surface water, bottled mineral water, and different sites within a drinking water treatment plant, including distribution system and tap water, to degrade sulfamethoxazole (SMX).

#### Methods

From a total of 133 isolates, were selected 51 representative strains affiliated to 19 genera - *Pseudomonas*, *Sphingomonas*, *Blastomonas*, *Acinetobacter*, *Aeromonas*, *Variovorax*, *Microbacterium*, *Duganella*, *Bosea*, *Afipia*, *Brevundimonas*, *Acidovorax*, *Rhizobium*, *Klebsiella*, *Citrobacter*, *Raoultella*, *Enterobacter*, *Kluyvera*, and *Stenotrophomonas* to test the ability to degrade SMX. The biodegradation experiments were carried out in mineral and complex culture media supplemented with 50 mg/L SMX.

#### Conclusions

Five isolates belonging to the genus *Pseudomonas* and one to the genus *Stenotrophomonas* were able to degrade SMX in complex culture medium. *Pseudomonas mandelii* isolated from bottled mineral water and *Stenotrophomonas maltophilia* isolated from river water were the most efficient SMX-degrading bacteria, removing 67% and 38% of the initial SMX content after 15 days of incubation, respectively.

Based on the potential application of these microorganisms or catalysts thereof, further research on the SMX degradation kinetics and metabolites are under study and will be discussed with what is described for other *Proteobacteria* and *Actinobacteria*.

## **FEMS-2053**

### **Antibiotic resistance and environment**

#### **EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL) PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE STRAINS IN THE HUMAN MICROBIOTA BIOBANK (HUMB) AT THE DEPARTMENT OF MICROBIOLOGY, UNIVERSITY OF TARTU**

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#### **Background**

Extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* is an increasing public health problem. Extensive collection of these strains is needed to analyse the issue more thoroughly. Human Microbiota Biobank (HUMB) at University of Tartu ([www.eemb.ut.ee/eng/](http://www.eemb.ut.ee/eng/), WFCC-MIRCEN no. 977) is a scientific collection that contains beneficial and opportunistic microbial strains of human origin.

#### **Objectives**

To form the subcollection of ESBL positive *Enterobacteriaceae* strains in HUMB for further pheno- and genotypic characterisation.

#### **Methods**

More than 1200 strains of ESBL positive *Escherichia coli* and *Klebsiella pneumoniae* were collected from 20 hospitals in Estonia (n=627), Latvia (n=182), Lithuania (n=134) and St. Petersburg region in Russia (n=322) in 2011-2012. The strains were identified by MALDI-TOF MS and deposited at the HUMB collection (stored at -80°C in multiple parallels in multiple freezers). Bacterial isolates were compared according to clinical material, geographical origin, phylogenetic group, antibiotic susceptibility and virulence factor genes.

First results of the subcollection of ESBL producing strains have been published (Pavelkovich 2014; Lillo 2014). The regional differences of virulence factor genes and carbapenemase resistance remained statistically significant after taking into account the phylogenetic distribution in the countries. MALDI-TOF MS-based assay appeared to be suitable, quick and cost-effective method for the initial confirmation of carbapenemase production.

#### **Conclusions**

Subcollection of ESBL producing strains stored in HUMB have significant scientific value that has to be further studied for epidemiological purposes and for creating evidence-based suggestions for clinicians.

## **FEMS-1020**

### **Antibiotic resistance and environment**

#### **IMPACT OF AOP ON ANTIBIOTIC RESISTANT BACTERIA AS PART OF THE WASTEWATER EFFLUENT MICROBIOM**

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Wastewater treatment plants are one source of microbial contamination. In the light of recent studies there are reports of increased levels of multi-resistant bacteria and antibiotic resistance genes (ARGs) in wastewater treatment plants (WWTPs) and downstream habitats. Conventional WWTPs represent a reservoir for the dissemination of ARGs in the bacterial communities. Horizontal gene transfer (HGT) of antibiotic resistance genes contributes to increased abundances of hygienically relevant, antibiotic resistant bacteria in aquatic systems. Advanced oxidation processes (AOPs) as additional wastewater treatment techniques are investigated to decrease the bacterial load and the dissemination of antibiotic resistances in adjacent downstream aquatic habitats.

To investigate the efficiency of ozonation to reduce the bacterial load, the project includes detection and quantification of facultative pathogens and medically relevant antibiotic resistance genes in microbial communities by qPCR analysis.

We investigate the impact of ozonation by molecular quantification of the methicillin resistance gene *mecA* from staphylococci, the ampicillin resistance gene *ampC* from Enterobacteriaceae, the transposon mediated vancomycin resistance gene *vanA* from enterococci, the imipenem resistance gene *blaVIM* from *Pseudomonas aeruginosa*, and the erythromycin resistance gene *ermB* from streptococci.

As expected, most of the hygienically relevant bacteria were reduced during ozonation, but it became obvious that bacteria carrying antibiotic resistance genes showed a considerable robustness and fitness against ozonation. This indicates a selection-like process favoring already antibiotic resistant bacteria during oxidative wastewater treatment. Further investigations regarding drug-dependent predispositions and different bacterial stress response mechanisms are currently analyzed to characterize the bacterial adaptation.





**FEMS-0683**

**Antibiotic resistance and environment**

**RESISTANCE TO AZTREONAM IN ENVIRONMENTAL ISOLATES OF PSEUDOMONAS AERUGINOSA RESULTS FROM HYPEREXPRESSION OF MEXAB-OPRM INDUCED BY MUTATION IN NALC GENE.**

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**Background**

Bacteria belonging to the genus *Pseudomonas* have a great metabolic versatility and acquire resistance to a wide variety of antimicrobials. Resistance to the monobactam aztreonam occurs primarily by changing in the efflux pump MexAB-OprM system. Three transcriptional regulators modulate the expression of this operon: MexR, from MarR family, and NalD and NalC, TetR family members. NalC is an anti-repressor that allosterically inhibits the MexR dimer formation and, then, MexR is released from the mexAB-oprM promoter, leading to an upregulation of this operon.

**Objectives**

This study aimed to determine the resistance profile to aztreonam in 50 *P. aeruginosa* isolates and to determine if mutations in the regulatory genes affected the expression of MexAB-OprM efflux pump.

**Methods**

The soil sample was collected and processed according to the method describe by Mukherjee et al. (2010), and the resistance profile was determined by the Minimum Inhibitory Concentration (MIC) method. The transcriptional regulators *mexR*, *nalC* and *nalD* were amplified by PCR, and the expression of MexAB-OprM was measured by quantitative real-time reverse transcriptase-PCR (qRT-PCR).

**Conclusions**

All isolates analyzed had some amino acid substitution in NalC, only one isolate showed a substitution at MexR and no single mutation was detected into NalD protein. Just the isolate that presented mutation at position 186 had MIC more than 250 µg mL<sup>-1</sup> and showed a greater increase in the level of *mexA* expression, about 92.6 fold more than that found in the wild-type PAO1 strain.

## **FEMS-3052**

### **Antibiotic resistance and environment**

#### **GENETIC DIVERSITY AND ANTIMICROBIAL RESISTANCE OF PSEUDOMONAS AERUGINOSA ISOLATED FROM HUMAN, VETERINARY AND ENVIRONMENTAL SAMPLES IN ESTONIA**

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#### **Background**

*P. aeruginosa* is an opportunistic microorganism widely spread in different terrestrial and aquatic environments, but it is also known multiresistant pathogen in hospital setting.

#### **Objectives**

We aimed to characterize the population structure of *P. aeruginosa* isolated from human, animal and environmental samples; to identify the potential transfer routes between various environments and to look for the associations between antibiotic resistance and potential resistance genes.

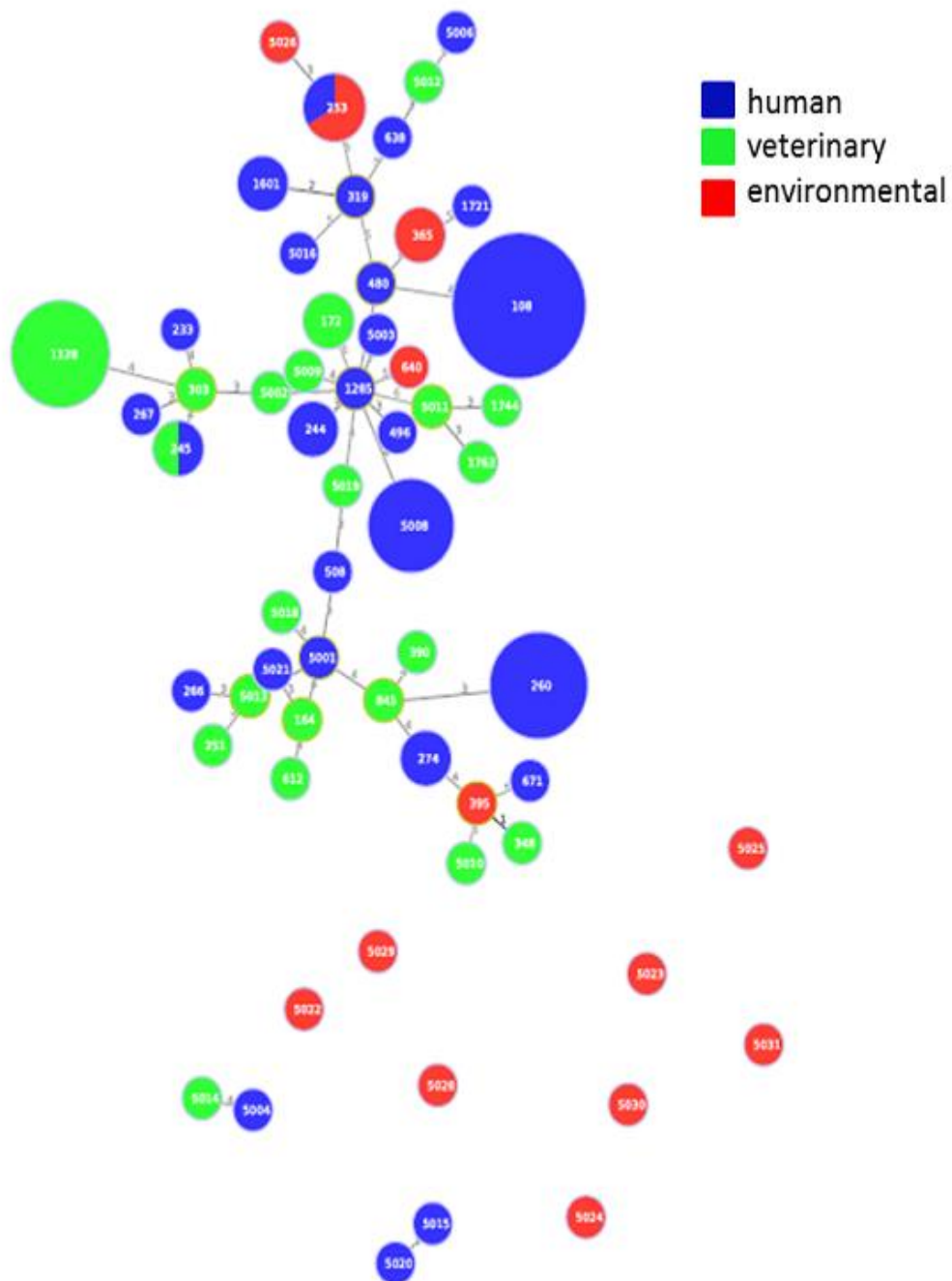
#### **Methods**

Ninety strains were collected between 2004 and 2014: 41 clinical isolates from patients with infection; 4 colonising strains from nasal and faecal samples of 287 healthy volunteers; 29 isolates from sick animals and 16 from environmental samples.

Study has been approved by Research Ethics Committee of the University of Tartu.

All samples underwent whole genome sequencing. Draft genomes were assembled followed by *in silico* identification of markers for multilocus sequence typing (MLST)

analysis. Genetic relatedness was presented as minimum spanning tree.



**Minimum spanning tree of *P.aeruginosa* from various sources.**  
Each circle corresponds to a unique sequence type from MLST database ([pubmlst.org/paeruginosa](http://pubmlst.org/paeruginosa)).  
Circle size is proportional to sequence type frequency

Coding regions for beta-lactamases were predicted from draft genomes using custom HMM profiles for Ambler class A, D, C and B beta-lactamases.

MICs to 10 antipseudomonal agents (ceftazidime, cefepime, meropenem, imipenem, piperacillin/tazobactam, amikacin, gentamycin, tobramycin, ciprofloxacin, colistin) were detected using epsilon meter test. EUCAST breakpoints and multiresistance definition were used for interpretation.

Phenotypic resistance was compared to the betalactamase genes predicted from the genomic sequences.

## **Conclusions**

Our data indicates that *P. aeruginosa* strains are transferred between different hosts and environments. The high variety of phenotypic resistance and resistance genes in bacteria of the same sequence type suggests rapid horizontal transfer of resistance.

## **FEMS-2909**

### **Antibiotic resistance and environment**

#### **AMINOGLYCOSIDES AND PENICILLINS: ASSESSMENT OF “IN VITRO” SYNERGY FOR ENTEROCOCCUS SP – DISPARITY BETWEEN CLINICAL PRACTICE AND “IN VITRO” RESULTS**

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#### **Background**

Developing dosage guidelines for antibiotics with mitigation of adverse risks is a goal of the rational use of medications. The use of checkerboard tests enables evaluation “in vitro” of the association of antibiotics in different concentrations and optimization of gentamicin and ampicillin concentrations in terms of therapeutic effectiveness, contributing to more effective therapeutic treatment guidelines.

#### **Objectives**

The objective of the study is to evaluate the synergism of action of gentamicin associated to ampicillin through “in vitro” studies in enterococci strains.

#### **Methods**

A total of 50 *Enterococcus sp* strains were used and the susceptibility profile to ampicillin and gentamicin evaluated separately and in combination. For each enterococci strain, a total of 36 tests were performed in 1 mL tubes with different concentrations of ampicillin and gentamicin. Figure 1.

#### **Conclusions**

A total of 1,800 tests were conducted with different concentrations of antibiotics. After 24 hours and 48 hours of incubation, the turbidity, representing the action of antimicrobial on the enterococci strains, was observed.

The synergism of action was observed in 10% of samples, with 54% indifference and 32% revealing therapeutic treatment failure. In other words, mere association of the antibiotics does not result in successful therapeutic treatment. Evaluation “in vitro” of different concentrations of antibiotics can help guide proper treatment of infections through appropriate doses that permit synergy of action and therapeutic success.

Figure 1: Checkerboard – Evaluation of the synergism of action between different concentrations of ampicillin and gentamicin on enterococci strains,  $1.5 \times 10^8$  UFC/mL.

The initial dose of antibiotics was 0.4mg/mL, corresponding to 10µg discs with progressive dilution of the antibiotics.

	Ampicillin (mg/mL)						
Gentamicin (mg/mL)		0.4	0.2	0.1	0.05	0.025	0.0
	0.4	Negative Control					
	0.2						
	0.1						
	0.05						
	0.025						
	0.0						Positive Control

## **FEMS-2912**

### **Antibiotic resistance and environment**

#### **CORRELATING METAL CONTENT IN (EXTREMELY) POLLUTED SOIL WITH BACTERIAL RESISTANCE TO ANTIBIOTICS AND METALS**

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#### **Background**

Microbes require some (trace) metals for growth, while high metal concentrations can be toxic. Metal-resistant microbes have evolved in metal-polluted environments, some of which may also have (co-)developed antibiotics resistance.

#### **Objectives**

This study aims to determine the environmental risk of (spread of) antibiotic resistance to humans from historically metal-contaminated urban areas.

#### **Methods**

This study analysed the spatial variation of the concentrations of 16 metals in 375 topsoil samples from the Swansea/Neath/Port Talbot urban area, which contain some of the highest metal-polluted soils of the UK. Pairwise correlations showed mostly significant positive associations between 16 of the studied elements, e.g. copper and arsenic. Factor analysis showed that 68% of the observed variation in concentrations could be explained by four principal components. The archived soils were then subsampled over a range of metal concentrations, yielding 65 subsamples for further biomolecular analysis.

#### **Conclusions**

Culture-dependent studies on dilute nutrient rich and humic acid agar media yielded many isolates with medium to high tolerance to several metals and antibiotics. Culture-independent studies are performed to determine the bacterial diversity and abundance of resistance genes over the wide range of observed (mixed) metal concentrations.

## **FEMS-1907**

### **Antibiotic resistance and environment**

#### **THE HUMAN GUT AS A RESERVOIR FOR ANTIBIOTIC RESISTANCE GENES**

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#### **Background**

The human gut harbors a densely populated microbial ecosystem, termed the gut microbiota. The gut microbiota can serve as a reservoir for antibiotic resistance genes ('the resistome') and drug-resistant opportunistic pathogens.

#### **Objectives**

In this study, we determined the dynamics of the microbiota and the resistome in thirteen patients that were hospitalized in Intensive Care Units. Because gut-associated bacteria and their resistance genes may be shed into the environment through sewage, we also assayed the levels of antibiotic resistance genes in hospital sewage and in subsequent stages of wastewater treatment.

#### **Methods**

16S rRNA profiling, functional metagenomics, metagenomic sequencing and nanolitre-scale quantitative PCRs were used in this study.

#### **Conclusions**

The gut microbiota of hospitalized patients contained genes that are predicted to confer resistance to fourteen classes of antimicrobials. Levels of antibiotic resistance genes increased during ICU hospitalization and were associated with changing patterns of the composition of the microbiota. Sequencing of vector inserts in drug-resistant fosmid clones indicated that resistance genes were mainly carried by anaerobic gut commensals (*Clostridium* and *Bacteroides*). Resistance genes were associated with mobile genetic elements, which could facilitate horizontal gene transfer of resistance genes from commensal bacteria to opportunistic pathogens. Antibiotic resistance genes were abundant in hospital sewage but the influence of hospital sewage on the abundance of resistance genes in the wastewater treatment



plant was limited. Levels of resistance genes and human-associated bacteria further decreased during wastewater treatment, limiting the dispersal of antibiotic resistance genes from hospitals into the environment.

## FEMS-2149

### Antibiotic resistance and environment

#### CLINICALLY-RELEVANT QUINOLONE RESISTANT ESCHERICHIA COLI ARE UBIQUITOUS IN THE ENVIRONMENT

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#### Background

Molecular epidemiology-based studies using methods such as multilocus sequence typing (MLST) are useful to evaluate the dissemination of antibiotic resistant bacteria and their genes (ARB&ARG) in the environment. If the ARB bacteria tracked are typical human commensals like *Escherichia coli*, it is also possible to infer about the humans-environment relationship.

#### Objectives

This study aimed at assessing the genetic relatedness of quinolone unsusceptible *E. coli* isolated from different types of water, gulls, birds of prey and hospitalized patients, in order to infer about possible modes of transmission of ARB in the environment.

#### Methods

A group of 143 quinolone unsusceptible *E.coli* from clinical, wild birds and water isolates was compared based on MLST and resistance phenotype. Water isolates were further analyzed regarding the presence of chromosomal mutations in the genes *gyrA* and *parC*; of plasmid-mediated quinolone resistance (PMQR, *qnrA*, *B*, *C*, *D*, *S*, *VC*; *qepA*; *oqxAB*; *aac(6')-ib-cr*)- and beta-lactam resistance (*bla*<sub>OXA-A,B,TEM,CTX,SHV,CMY</sub>)-encoding genes; and of Inc group plasmid replicons. Water isolates carrying PMQR-encoding genes were tested for their ability to transfer resistance by conjugation.

#### Conclusions

Isolates from different sources clustered together, suggesting the ability to persist and spread across different environments. The most common sequence types were ST131 (n=26) and ST10 (n=9). 77% of the isolates were resistant to three or more classes of antibiotics. Eight water isolates harbored PMQR-encoding genes, combined with *bla*<sub>TEM</sub> (n=6) and *bla*<sub>CTX-M-15</sub> (n=2), from which seven could transfer

resistance through conjugation. These results confirm the role of multidrug-resistant *E.coli* in the dissemination of ARB&ARG across different environments.

**FEMS-2152**

**Antibiotic resistance and environment**

## **AEROMONAS SPP. AS CARRIERS OF ANTIBIOTIC RESISTANCE GENES IN HOSPITAL AND MUNICIPAL EFFLUENTS**

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<sup>2</sup>LEPABE - Laboratory for Process Engineering Environment Biotechnology and Energy, Faculty of Engineering University of Porto, Porto, Portugal

### **Background**

Over the last years, members of the genus *Aeromonas* have been recognized as one of the principal reservoirs of antibiotic resistance in aquatic environments. While several studies have documented the levels of antibiotic resistance associated with these bacteria in urban waterways, the contribution of hospital effluents remains unclear.

### **Objectives**

The objective of this study was to elucidate the role of this genus in the dissemination of antibiotic resistance from hospital effluent into the environment.

### **Methods**

A set of quinolone unsusceptible *Aeromonas* was isolated from untreated hospital effluent and from the inflow and treated effluent of the receiving urban wastewater treatment plant (UWTP). Isolates were identified and genotyped based on the 16S rRNA, *cpn60* and *gyrB* gene sequences. Further characterization involved determination of the antibiotic resistance phenotypes and screening for antibiotic resistance and mobile genetic elements (*qnrA,B,C,D,S,VC*; *qepA*; *oqxAB*; *aac(6')-ib-cr*; *blaOXA*; *incU*).

### **Conclusions**

The most prevalent taxonomic groups were *A. hydrophila* and *A. caviae*, found in all types of water, while *A. salmonicida* was found exclusively in the hospital effluent and raw UWTP inflow, and *A. allosaccharophyla* and *A. veronii* in the UWTP. The genetic determinants *qnrS2+incU* were a signature of the UWTP and almost absent in the hospital effluent. The genes *aac(6')-ib-cr* and *blaOXA* were distributed in isolates from all sources. The dynamics of resistance genes and mobile genetic elements was evidenced by the fact that closely related isolates harbored different combinations of those genetic elements. This observation highlights the role of *Aeromonas* as important carriers of antibiotic resistance genes in aquatic environments.

## **FEMS-1334**

### **Antibiotic resistance and environment**

#### **CULTIVABLE GRAM-NEGATIVE BACTERIA IN DRINKING WATER: UBIQUITY, PERSISTENCE AND ANTIMICROBIAL RESISTANCE**

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#### **Background**

To achieve required standards of quality and safety, in most world regions, drinking water must undergo purification and disinfection processes prior to its distribution. However, tap water is colonized by a myriad of bacteria comprising those that survive disinfection or that enter at any stage of the distribution network. Despite the multiple factors affecting water microbiota, some bacterial groups are known to prevail in drinking water<sup>1</sup>. Good examples are given by genera of *Alpha*-, *Beta*- and *Gamma-Proteobacteria*, some of which display high levels of antibiotic resistance.

#### **Objectives**

The objective of this study was to characterize the diversity of cultivable Gram-negative bacteria isolated from tap water, collected over nine months. The aim was to assess which were the most prevalent, ubiquitous and persistent groups in drinking water and to evaluate their antimicrobial resistance potential.

#### **Methods**

More than 3000 isolates recovered from the water source, the water treatment and distribution system, and from 12 household taps, were screened for Gram-negative staining (51%) and absence of repetitions (same plate and identical genotype). From this selection resulted approximately 1100 isolates that were, most of which, identified by 16S rRNA or *rpoB* gene sequence analysis. The most representative groups occurring in tap water were characterized for their antimicrobial resistance phenotypes.

#### **Conclusions**

Among most ubiquitous groups were the genera *Blastomonas*, *Brevundimonas*, *Methylobacterium*, *Sphingobium*, *Sphingomonas*, *Acidovorax*, *Ralstonia*, *Acinetobacter* and *Pseudomonas*. Some of these groups comprise opportunist pathogens and present profiles of multidrug resistance, making their presence in treated drinking water an issue of concern.

Reference:<sup>1</sup>Vaz-Moreira *et al.*, FEMS Microbiol Rev 38(4),761-778.

**FEMS-1377**

**Antibiotic resistance and environment**

## **MULTIDRUG RESISTANT GRAM-NEGATIVE BACTERIA FROM HOSPITAL EFFLUENT**

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### **Background**

Hospital effluents are frequently discharged untreated in municipal collectors. However, this type of wastewater deserves a special attention given the high prevalence, and potential emerging character, of antibiotic resistance found in clinical environments.

### **Objectives**

This study aimed at identifying quinolone resistant Gram-negative bacteria with the highest multidrug resistance index (MDR) thriving in hospital effluent or in the receiving municipal wastewater treatment plant. It was aimed to assess if these bacteria may represent critical vectors for the spread of antibiotic resistance from the hospital to the environment.

### **Methods**

The criteria for inclusion in this study was to be Gram-negative, other than *Escherichia coli* and present reduced susceptibility to quinolones, a MDR phenotype (to  $\geq 6$  classes of antibiotics) or of resistance to meropenem or ceftazidime (n=25). These potential vectors of resistance were identified and characterized for antibiotic resistance phenotype, plasmid mediated quinolone resistance (PMQR) genes, presence of integron gene cassettes and conjugative capacity.

### **Conclusions**

MDR isolates comprised bacteria of the genera *Aeromonas*, *Acinetobacter*, *Citrobacter*, *Enterobacter*, *Klebsiella*, *Pseudomonas*, *Chryseobacterium* and *Myroides*. About half of these isolates had at least one PMQR gene (*aac(6')*-*Ib-cr*, *qnrB*, *qnrS* or *oqxAB*) and a class 1 integron gene cassette encoding aminoglycoside, sulfonamide or carbapeneme resistance. Some isolates, mainly *Enterobacteriaceae*, could transfer through conjugation some resistance traits. These results showed that ubiquitous bacteria, other than the groups whose monitoring in water quality controls is recommended, are important vectors of antibiotic resistance. This information is useful to support management options aiming the control of this public health problem.

## **FEMS-1832**

### **Antibiotic resistance and environment**

#### **COUNTERACTING ANTIBIOTIC RESISTANCE IN AGRICULTURE: BIOTECHNOLOGICAL BARRIERS MIGHT DO THE TRICK**

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#### **Background**

The application of untreated manure and dung to agricultural areas provides essential fertilization for crops and pasture. However, it enables the entry of veterinary pharmacological compounds like antibiotics and heavy metals into the environment, thus promoting the distribution of resistant and potentially pathogenic microorganisms in soil, as well as ground and surface waters.

#### **Objectives**

The aim is to assess the risk of untreated agricultural wastes containing pathogens and antibiotic resistance genes on environment and human health while ascertaining the beneficial effects of manure treatment through fermentation.

#### **Methods**

Within a BMBF joint research project, we were able to monitor input and output samples of 15 German biogas plants, compiling extensive data on the chemical and microbiological status. Key organisms (e. g. Enterobacteriaceae, Enterococcaceae, Clostridiaceae) of every sample were genetically identified and archived in a strain collection to be tested for resistance and screened for tetracycline and sulphonamide resistance genes. Additionally, 4 resistance genes were quantified in all total DNA extracts and monitored in laboratory bioreactors under the influence of antibiotic supplementing over the course of 2 months.

#### **Conclusions**

Micro- and molecular biological approaches indicate a reduction of aerobic cultivable bacteria through fermentation including pathogens of the Enterobacteriaceae and Enterococcaceae without promotion of anaerobic and spore forming pathogens e.g. *Clostridium spp.* Although antibiotic resistance genes were found ubiquitously in both manure and digestate, a decline in resistance activity and resistance gene quantity after fermentation could be detected. Ultimately, biogas fermentation presents a

promising strategy to limit environmental pollution and risks for human health caused by agricultural wastes.



**FEMS-2219**

**Antibiotic resistance and environment**

**WIDESPREAD DISTRIBUTION OF MULTIDRUG RESISTANCE IN COMMUNITY ASSOCIATED STAPHYLOCOCCI**

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**Background**

Staphylococci are opportunistic pathogens that have emerged as a major cause of nosocomial infections. Methicillin resistance is associated with the *mecA* gene, which is located on a mobile genetic element called staphylococcal cassette chromosome *mec*(SCC*mec*). A new *mecA* homolog designated as *mecA*<sub>LGA251</sub> has been described recently.

**Objectives**

The purpose of this study was to evaluate multidrug resistant staphylococci recovered from hands of the general public.

**Methods**

267 staphylococci isolates were isolated from the general public in London, UK. All isolates were cultured, identified using MALDI-TOF-MS and tested for susceptibility to 13 antibiotics using disk diffusion method. In addition, MICs to oxacillin were also determined. *mecA* and *mecC* genes and SCC*mec* types were determined using PCR.

**Conclusions**

267 isolates belonging to 10 different staphylococcal species were recovered in this study. 223 (83.5%) staphylococcal isolates were resistant to more than two antibiotics. MICs to oxacillin varied from 0.06 mg/L to 256 mg/L. Seven out of 223 staphylococcal isolates were *mecA* positive and 3 *mecC* positive. SCC*mec* types were assigned for 4 out of 10 *mecA* and *mecC* positive strains. 2 SCC*mec* types V and 1 type VI were *mecA* positive and 1 SCC*mec* type IV was *mecC* positive.

We report on the widespread distribution of antibiotic resistant strains on the hands of the general public. We also report for the first time the isolation of *mecA* homologue *mecC* gene from randomly selected individuals. This emphasises the importance of good hygiene within the community.

**FEMS-0977**

**Antimicrobial resistance**

**EVALUATION OF CLASS 1-3 INTEGRONS AMONG PSEUDOMONAS AERUGINOSA CLINICAL ISOLATES IN MILAD HOSPITAL, TEHRAN, IRAN**

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**Background**

*Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen that is found in soil, water and moist environments. *P. aeruginosa* infections are major cause of hospital morbidity and mortality.

**Objectives**

The current study aimed to demonstrate the frequency of class 1, 2 and 3 integrons in *P. aeruginosa* clinical isolates collected from urinary tract infections in Milad Hospital, Tehran, Iran.

**Methods**

Sixty *P. aeruginosa* strains were isolated from urinary tract infections during period December to March 2012. Antibiotic susceptibility testing was performed by disk diffusion method according to CLSI. Then, DNA of isolates were extracted and the presence of class 1, 2 and 3 integrons were examined by PCR.

**Conclusions**

**Results:** The antibiotic susceptibility pattern of *P. aeruginosa* clinical isolates were as follows: resistance to cefepime, ceftriaxone, Aztreonam, gentamicin, amikacin, ciprofloxacin, ofloxacin, ceftazidime, piperacillin tazobactam, and imipenem were 73.3%, 40%, 51.6%, 31.7%, 26.6%, 43.3%, 45%, 35%, 23.3%, and 15%, respectively. Based on our analysis, 73.3% and 1.6% of *P. aeruginosa* clinical isolates were positive int-1 and int-2, respectively. The negative results obtained for int-3.

**Conclusion:** Our findings demonstrated high frequency of class I integron in *P. aeruginosa* clinical isolates and therefore, the class I integron might play a special role in transfer of antibiotic resistant genes

**FEMS-0492**

**Antimicrobial resistance**

**PHENOTYPIC DETECTION OF EFFLUX PUMPS IN MULTI-DRUG RESISTANT PSEUDOMONAS AERUGINOSA ISOLATED FROM SUEZ CANAL UNIVERSITY HOSPITAL**

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**Background**

Infections caused by *Pseudomonas aeruginosa* are often severe, life threatening and difficult to treat because of the limited susceptibility to antimicrobials, the high frequency of emergence of antibiotic resistance during therapy and the emergence of multi-drug resistant (MDR) strains that develop resistance by various mechanisms including efflux pumps. Efflux pumps contribute to multidrug resistance by expelling antibiotics out of cells.

**Objectives**

Detection of the role of efflux pumps in MDR *Pseudomonas aeruginosa* isolates from Suez Canal university hospital in Ismailia.

**Methods**

The study included 49 *Pseudomonas aeruginosa* strains isolated from 307 nosocomially infected patients. Antibiotic susceptibility tests were done by disc diffusion method. For detection of association of efflux pumps, strains proved to be MDR were further tested for minimal inhibitory concentration (MIC), using the agar dilution susceptibility method, of four antibiotics of different groups (ciprofloxacin, meropenem, cefotaxime and gentamicin) before and after addition of the efflux pump inhibitor carbonyl cyanide m-chlorophenyl hydrazone (CCCP).

**Conclusions**

Using disc diffusion, 28 strains (57.1%) were proved to be MDR. The efflux pumps-associated resistance was proved through reduction of MIC in 18 (64.3%) , but not in 10 (35.7), of the MDR strains for ciprofloxacin and meropenem: 7 strains for ciprofloxacin, 7 for meropenem and 4 strains for both drugs together. This approximately twice association suggests a role of efflux pumps in MDR *Pseudomonas aeruginosa*. Understanding the mechanisms by which these pumps

act and how to overcome its activity opens the door for restoring the antibiotic activity and constitute a promising target for novel antibacterial agents.

## FEMS-2214

### Antimicrobial resistance

#### FIRST DESCRIPTION IN EUROPE OF A CIPROFLOXACIN RESISTANT *SALMONELLA* TYPHIMURIUM HARBOURING AAC6'-IB-CR AND OQXAB IN AN INCHI2 PLASMID

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#### Background

Fluoroquinolones are critical antibiotics (AB) for the treatment of *Salmonella* non-typhoid infections. Plasmid-mediated quinolone resistance (PMQR) genes have been often associated with decreased susceptibility to these AB worldwide, but concomitant presence of *oqxAB* and *aac6'-lb-cr* genes, increasingly described in Asia, is still scarce in Europe.

#### Objectives

To characterize the genetic background (clonality, AB resistance genes, their transferability and genetic environment) of ciprofloxacin resistant *S.Typhimurium* clinical isolates.

#### Methods

Two clinical isolates of *S.Typhimurium* resistant to ciprofloxacin [young children (feaces)/one hospital/2012/Portugal] were studied. Susceptibility to AB and detection of ESBL were assessed by disk diffusion and/or Etest methods (CLSI/EUCAST). Characterization of AB resistance genes, including topoisomerases (*gyrA/gyrB/parC/parE*) and PMQR [*qnrA/qnrB/qnrC/qnrD/qnrS/qepA/aac(6')-lb-cr/oqxAB*] genes, and metals (copper/silver/mercury/arsenic/tellurite) tolerance genes was performed (PCR/sequencing). Clonality (*Xba*I-PFGE/MLST), class 1 integron, plasmid characterization (PCR/sequencing), conjugation/transformation assays and genomic location (*I-Ceu*II/S1-PFGE hybridization) were also performed.

#### Conclusions

Presence of multidrug-resistant IncHI2-~180Kb plasmid encoding *oqxAB* and *aac-6'-lb-cr* in a ciprofloxacin resistant *S.Typhimurium*/ST34, also carrying *gyrA* mutation (Asp87Asn), is here firstly described in Europe. Co-resistance to nalidixic acid,

chloramphenicol-*cmlA/floR/catB3*, gentamicin-*aac(3)-IV*, kanamycin-*aphA1*, streptomycin-*aadA/strA-strB*, sulfamethoxazole-*sul1/sul2/sul3*, tetracycline-*tetB*, trimethoprim-*dfrA12*, ampicillin-*bla<sub>TEM</sub>/bla<sub>OXA-1</sub>* and genes coding for metals tolerance (*merA/silA/pcoD/terF*) were found. Transformation of PMQR carrying plasmid was associated with resistance acquisition to several antibiotics (*cmlA/floR/catB3/aac(3)-IV/aphA1/aadA/sul1/sul2/sul3/dfrA12/bla<sub>OXA-1</sub>*) and an eight-fold increase on ciprofloxacin MIC. Interestingly, the IncHI2 plasmid carried *oqxAB* genes flanked by IS26 and an incomplete class 1 integron containing *aac-6'-lb-cr-bla<sub>OXA-1</sub>-catB3-arr3-qacEΔ1-sul1*, atypical *sul3*-integron and *terF*. A similar PMQR genetic environment in *Salmonella* with IncHI2 circulating in Asia was observed, highlighting the role of these plasmids in *oqxAB* and *aac-6'-lb-cr* global spread.

**FEMS-0512**

**Antimicrobial resistance**

**ASSESSMENT OF MULTIPLE ANTIBIOTIC RESISTANT ENTEROCOCCI IN COMMERCIAL AND COMMUNAL CATTLE FAECES AND THEIR WATER SOURCES IN MAFIKENG, NORTH-WEST PROVINCE, SOUTH AFRICA**

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**Background**

Enterococci are part of the intestinal microflora of most mammals.

**Objectives**

To isolate and determine the antibiotic resistance profiles of *Enterococcus* species from cattle farms in South Africa

**Methods**

A total of 49 faecal and 16 water samples from 2 commercial farms (Molelwane and Rooigrond) and a communal farm (Gelukspan) were analysed for *Enterococcus* species. 138 potential isolates were obtained and 129 enterococci were positively identified. All the 129 isolates were subjected to antibiotic susceptibility tests. The antibiotic inhibition zone diameter data of the isolates were subjected to cluster analysis and the results expressed as dendrograms. Vancomycin and tetracycline specific PCR was also performed.

**Conclusions**

*Enterococcus avium* (61%) and *Enterococcus faecium* (32%) were the dominant species isolated from both diarrhoeal and healthy cattle compared to *Enterococcus durans* (5%) and *S. bovis* (2%). Only *Enterococcus avium* (13.9%) and *Enterococcus faecium* (10.1%) were isolated from water samples. Large proportion (30%) of the isolates were resistant to tetracycline compared to vancomycin (17%). The MAR phenotype GM-SMX-NAL-NIT-C-K-S was dominant (38%) among *E. faecium* isolated from communal and commercial cattle while GM-SMX-NAL-NIT-K-S was dominant among *E. faecium* (40%), *E. durans* (60%) and *E. avium* (42%) isolated healthy and diarrhoeal cattle in both the communal and commercial farms. None of the enterococci isolates were positive for the *vanA* and *vanB* genes but the *vanC* gene was detected in 20% and 1.6% of *E. durans* and *E. avium*, respectively. Cluster analysis could not distinguish between isolates from commercial and communal farms indicating that these isolates had similar antibiotic resistant profiles.

**FEMS-1443**

**Antimicrobial resistance**

**MOROCCAN BIOACTIVE ACTINOBACTERIA ISOLATES PRODUCING EFFLUX PUMPS INHIBITORS (EPI) OF RESISTANT BACTERIA**

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**Background**

Traditional antimicrobials antibiotics are increasingly suffering from the emergence of multidrug resistance among pathogenic microorganisms. Among the antibiotic resistance mechanisms, efflux pumps have recently received a particular attention. Hence, there is an acute need for new active agents.

**Objectives**

Reducing the rate of emergence of antibiotic-resistant is our objective by studying the ability of Moroccan actinobacteria isolates to produce natural efflux pumps inhibitors (EPI) of medical interest

**Methods**

210 actinobacteria isolates were screened for their ability to produce efflux pumps inhibitors using agar diffusion method. As test strains we have used in this study the wild type strain *E. coli* AG100 and its mutant AG100A with non- functional pump efflux system and the two clinical strains of *S.aureus*: the sensitive one SA-1199 and its mutant SA1199B. Phe-Arg- $\beta$ -naphtylamide (PA $\beta$ N) which inhibits the efflux system of many bacteria was used as control. Finally the biological and chemical characterizations of active compounds were conducted from four liters fermentation culture of selected bioactive.

**Conclusions**

Our screening program showed that among the 210 screened actinobacteria isolates, 9% were able to produce EPI. From the most promising ones we have purified five different bioactive compounds and their structure elucidation were under investigation. Screening only a subsection of our natural product library led to purify five molecules as EPI capable of sensitizing Gram-negative and Gram positive bacteria to antibiotics to which they are ordinarily intrinsically resistant. This result demonstrates the great potential of this approach in expanding antibiotic effectiveness in the face of the growing challenge of resistance



## **FEMS-2292**

### **Antimicrobial resistance**

#### **IN VITRO ACTIVITIES OF SEVEN ANTIFUNGAL DRUGS AGAINST CANDIDA AFRICANA, AN ATYPICAL AGENT OF VULVOVAGINAL CANDIDIASIS**

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### **Background**

Vulvovaginal candidiasis (VVC) is a common vaginal fungal infection, affecting 70–75% of young women at least once during their lifetime. *Candida africana* is an opportunistic yeast pathogen that was proposed as a new species within the *C. albicans* species complex in 2001. It has been reported from 12 countries predominantly as an agent of VVC. Although, recently a large variety of antifungal drugs are used for treatment of VVC, treatment is a big challenge.

### **Objectives**

Therefore in the present investigation, we evaluated the in vitro antifungal susceptibility testing of 7 agents against *C. africana* isolated from vulvovaginitis patients

### **Methods**

5 strains out of 350 vaginal swabs were identified as *C. africana* based molecular methods. The MIC of AmB, FLU, ITC, VOR, POS, ISA, MICA, ANID were determined based on CLSI document M27-A3.

### **Conclusions**

The isolates were susceptible to Amphotericin B (MIC= 0.25 µg/ml), fluconazole (MIC= 1 µg/ml), itraconazole (MIC range, 0.5 - <0.016), voriconazole (MIC range, 0.5 - <0.016), posaconazole (MIC range, 0.5 - <0.016), isavuconazole (MIC range, 0.5 - <0.016), micafungin (MIC range, 0.016 - <0.008), anidulafungin (MIC range, 0.031 - <0.008).

*C. africana* has been largely associated with superficial infections of the genital tract, despite it has been associated with deep-seated infections, suggesting its invasive potential. The global patterns of resistance/susceptibility to most antifungal drugs remain largely unknown in *C. africana*; However high MIC of voriconazole, flucytosine and terbinafine against *C. africana* has been demonstrated. Antifungal susceptibility testing should be determined before therapy to avoid treatment failures.



## FEMS-2210

### Antimicrobial resistance

#### PROPIONIBACTERIUM ACNES IN BIOMATERIALS-ASSOCIATED INFECTION :FREQUENCY AND ANTIMICROBIAL RESISTANCE

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#### Background

*Propionibacterium acnes* can cause invasive deep seated infections, usually in the setting of implantable devices and its antimicrobial resistance is beginning to emerge.

#### Objectives

*P.acnes* can act as an opportunistic pathogen causing invasive and chronic biomaterials-associated infection through a biofilm mode of growth. In this study we aimed to review the frequency and the antimicrobial resistance of *P. acnes* in infections involving implants.

#### Methods

From March 2011 to April 2014, cerebrospinal fluid specimens (CFS) of 46 patients with internal shunt infection (ISI), corneal scrapings (CS) of 55 patients with delayed postoperative endophthalmitis (DPE) developed after lens implantation, periprosthetic tissues (PT) of 11 patients with prosthetic shoulder infection (PSI) and 17 patients with prosthetic joints infection (PJI) were studied. Samples were inoculated on supplemented Schaedler agars and fluid thioglycolate medium and were incubated during 15 days in anaerobic jars (Oxoid) on anaerobic conditions. Anaerobic bacteria were identified by API 20 A (BioMerieux). Susceptibility testing for penicillin, ampicillin+sulbactam, cefoxitin, clindamycin, tetracycline, rifampicin was performed with E-test (BioMerieux).

#### Conclusions

*P.acnes* was isolated from 2(4,3%) CFS, 16(29%) CS, 1(9 %) PT sample of patients with PSI and from 2(11.7%)PT samples of patients with PJI. These 21 *P.acnes* strains were susceptible to penicillin, ampicillin+sulbactam, cefoxitin and rifampicin whereas 38% (8/21) were resistant to clindamycin (MIC 90:32 mg/L), 14.2% (3/21) were resistant to tetracycline (MIC90: 4 mg/L). *P.acnes* is most frequently found in CS of patients with DPE developed after lens implantation. Its increasing clindamycin and tetracycline resistance should be taken into consideration during the treatment of biomaterials-associated infection.



## **FEMS-1004**

### **Antimicrobial resistance**

#### **ANTIBIOTIC SUSCEPTIBILITIES OF PSEUDOMONAS AERUGINOSA AND ACINETOBACTER BAUMANII IN A TERTIARY TRAINING HOSPITAL WITHIN EIGHT YEARS PERIOD**

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### **Background**

Gram negative non-fermenting bacteria related infections are among the major problems encountered in health care facilities. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are common and widely distributed non-fermenter pathogens which mostly cause opportunistic and nosocomial infections in patients at intensive care units as well as general wards.

### **Objectives**

In this retrospective study, we aimed to present antimicrobial susceptibility trends of common non-fermenters (*P.aeruginosa* and *A.baumannii*) in a tertiary referral hospital in Istanbul, Turkey. All chosen antimicrobial agents are consistently tested during study period, which covers eight years from 2006 to 2013.

### **Methods**

All *P.aeruginosa* (n:1312) and *A.baumannii* (n:1809) isolates from various specimens of inpatients from various clinics including ICUs were tested for antibiotic susceptibility for certain agents, between January 2006 and December 2013. Isolates were identified by conventional methods and/or commercial identification systems - API 20 NE (2006-2007) and VITEK 2 (2007-20013). Susceptibility testing of non-fermenters (n:3121) is performed by Kirby-Bauer disc diffusion method and results are interpreted according to CLSI guidelines.

### **Conclusions**

Multidrug resistance in *P.aeruginosa* and *A.baumannii* is a worldwide problem. The key point of our study is the demonstration of antibiotic susceptibilities of *P.aeruginosa* and *A.baumannii* isolates with more than 3000 isolates within a long period of eight years within our tertiary training hospital. Increasing carbapenem resistance is a major problem in our setting especially for *A.baumannii* strains. Among

tested aminoglycosides, tobramycin is found more effective than amikacin for *A.baumannii*. Quinolones were found quite effective against *P.aeruginosa in vitro* and even more effective than carbapenems for some years. But resistance rates were found very high against *A.baumannii*.

**FEMS-2143**

**Antimicrobial resistance**

## **FUSIDIC ACID AND MUPIROCIN RESISTANCE RATES AMONG STAPHYLOCOCCUS AUREUS CLINICAL ISOLATES**

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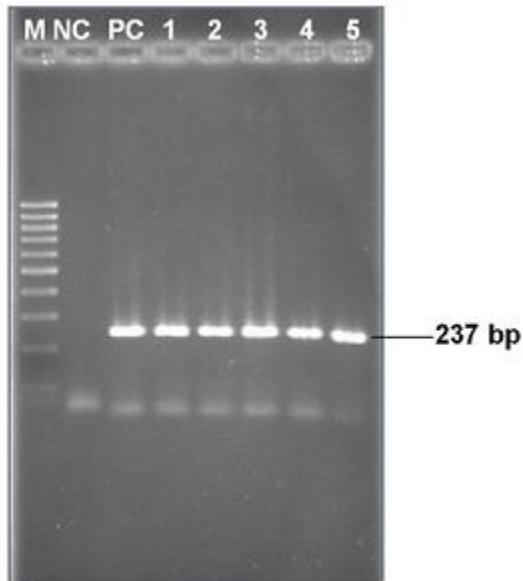
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### **Background**

*Staphylococcus aureus* is one of the major pathogens causing infections both in community and in health care settings. Both mupirocin and fusidic acid are topical antimicrobial agents that can be used for the treatment of staphylococcal skin infections and/or elimination of its nasal carriage.

### **Objectives**

We aim to determine high level (HL) mupirocin resistance and *in vitro* activity of fusidic acid in clinical *S. aureus* strains isolated between 2011 and 2013.



**Figure.** Gel electrophoresis of PCR products of *ileS2* gene. M: 100-bp DNA ladder; NC: negative control; PC: positive control; Lines 1, 2, 3, 4, 5 indicate *ileS2* positive isolates respectively.

### **Methods**

A total of 208 *S. aureus* strains, 86 methicillin resistant *S. aureus* (MRSA) and 122 methicillin susceptible *S. aureus* (MSSA), were tested for HL mupirocin with a 200 µg disc according to the CLSI guidelines. Fusidic acid resistance was investigated by 10 µg disc according to the criteria of EUCAST. HL mupirocin resistance was also confirmed by PCR for *ileS2* gene by using “*ileS2*-M1 5’ GTTTATCTTCTGATGCTGAG 3’ and *ileS2*-M2 5’ CCCCAGTTACACCGATATAA 3’” primers. Resistance to mupirocin and fusidic acid were found as 7.2% (15/208) and 17.8% (37/208). Resistance rates of mupirocin for MRSA and MSSA were detected

as 15.1% (13/86) and 1.6% (2/122). Resistance rates of fusidic acid for MRSA and MSSA were determined as 25.6% (22/86) and 12.3% (15/122).

### **Conclusions**

We detected lower resistance rates for mupirocin than fusidic acid among clinical *S. aureus* strains in our hospital. Today increased topical preparations and inappropriate clinical use of antibiotics seem major factors causing to resistance and clonal selection. Prescription of topical mupirocin or fusidic acid especially for prolonged treatment of skin infections and for decolonization of *S. aureus* from nares should be reconsidered.



## FEMS-2315

### Antimicrobial resistance

#### QUEST FOR NEW ANTIMICROBIAL DRUG TARGETS: GENOME-WIDE SCREENING OF ESSENTIAL GENES OF STREPTOCOCCUS SUIIS AND VALIDATION OF YYCF/G TWO-COMPONENT SYSTEM AS ANTIMICROBIAL DRUG TARGET

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#### Background

*Streptococcus suis* is a major bacterial pathogen of swine, causing high mortality and economic losses worldwide. *S. suis* is also emerging as an important cause of meningitis in humans in South East and East Asia. Antibiotic resistance of *S. suis* clinical isolates has been reported worldwide.

#### Objectives

Identification of novel antimicrobial drug targets of *S. suis* and validation of some of these targets.

#### Methods

Essential genes of *S. suis* S10 have been identified in a high-throughput genome-wide screen using a mariner transposon insertion approach (Tn-Seq). We constructed a list of *S. suis* S10 essential genes based on Tn-Seq results and published lists of orthologous essential genes of three Gram-positive bacteria. Based on this we selected several putative drug targets. We generated conditionally knock-out of *yycF/G* to elucidate its regulatory network and to validate its essentiality.

#### Conclusions

1) Genome-wide screening of a transposon insertion library of *S. suis* S10 revealed 281 essential genes of which 54 genes were shared with three other Gram-positive bacteria. 2) *yycF/G* was the only essential Two-component System in four selected Gram-positive bacteria. 3) A conditional knock-out strain of *yycF/G* in *S. suis* S10 validated its essentiality and potential as a drug target for novel antimicrobials.

## **FEMS-2010**

### **Antimicrobial resistance**

#### **ANTIFUNGAL RESISTANT YEAST FROM THE HARTS RIVER, SOUTH AFRICA**

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#### **Background**

Yeast species identified in a previous study in the North West Province are associated with superficial mucosal infections and life-threatening diseases. Treatment is by available antifungal drugs. Due to continuous exposure of aquatic yeast species to sub-therapeutic levels of antifungal agents, these species have developed resistance.

#### **Objectives**

The aim of this study was to determine the diversity and antifungal susceptibility patterns of yeasts in the Harts River of the North West Province, South Africa.

#### **Methods**

Yeast colonies were enumerated using yeast-malt-extract (YM) agar (containing 100 ppm chloramphenicol). Purified isolates from 37°C incubation were identified by biochemical tests and 26S rRNA gene sequencing. Disc diffusion antifungal susceptibility tests were conducted on the yeast species.

#### **Conclusions**

Yeasts levels ranged between 320-4200 cfu/L and 27-2573 cfu/L for room temperature and 37°C. All the yeast colonies isolated were non-pigmented. DBB tests determined the yeasts isolates as ascomycetes. Yeasts isolates were identified and belonged to the genera *Arxiozyma*, *Candida*, *Clavispora*, *Cyberlindnera*, *Lecythophora*, *Pichia*, *Saccharomyces*, and *Wickerhamomyces*. *Saccharomyces cerevisiae* and *Candida glabrata* were most frequently isolated species. A large number of isolates were resistant to azoles, especially fluconazole as well as other antifungal classes. Most of the *Candida* species were resistant to almost all the antifungals. Several of the isolated yeast species are opportunistic pathogens. These could cause infections in sensitive individuals during occasional direct contact. A major health concern is that these infections may not respond to available antifungal agents.

## FEMS-2231

### Antimicrobial resistance

#### C. PARAPSILOSIS AZOLE RESISTANCE CONFERRED BY A GAIN-OF-FUNCTION MUTATION IN MRR1 TRANSCRIPTION FACTOR

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#### Background

The human fungal pathogen, *Candida parapsilosis*, is mostly isolated from patients with bloodstream infections and its incidence in some countries is equal or exceeds that of *C. albicans*. For the treatment of these infections, azoles are widely used as prophylactic and therapeutic drugs. However, *C. parapsilosis* clinical azole resistance has been increasing in the last decade and little is known about the antifungal resistance mechanisms involved in the development of such resistance. Recently, voriconazole resistance mechanisms were associated with *MDR1* (encoding for a multidrug efflux pump) and *MRR1* (transcription factor) genes overexpression. Furthermore, a mutation in the *MRR1* gene (A2619C) that resulted in an alteration of lysine to asparagine, K873N, in polypeptide chain was firstly reported.

#### Objectives

Knowing that gain-of-function mutations found in transcription factors genes play a crucial role in azole resistance acquisition, we intend to elucidate the importance of A2619C mutation in *MRR1* gene in *C. parapsilosis* azole resistance.

#### Methods

For that, *MRR1*<sub>R<sub>VR</sub></sub> mutated gene was integrated in its native locus in a double deleted strain,  $\Delta mrr1/\Delta mrr1$ . Azole susceptibility and gene expression profile of the set of strains used in this study were characterized.

#### Conclusions

The *MRR1*<sub>R<sub>VR</sub></sub> complemented strain changed azole susceptibility profile, from susceptible to resistant, and also displayed an up regulation of *MRR1* and *MDR1* genes. Thus, missense mutation A2619C described in *MRR1* transcription factor is a

gain of function mutation, decisive for azole resistance.  
This study was supported by FEDER, COMPETE and FCT (PTDC/DTP-EPI/1660/2012).

## **FEMS-2234**

### **Antimicrobial resistance**

#### **SURVEILLANCE OF CANDIDA PARAPSILOSIS ANTIFUNGAL RESISTANCE**

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### **Background**

In Portugal, *C. parapsilosis* is the second most common yeast specie isolated from patients with bloodstream infections. However, *C. parapsilosis* increasing prevalence was been found in other European countries and in Latin America and Asia. Azoles are widely used as prophylactic and therapeutic drug; but *C. parapsilosis* acquires azole resistance in a rapid and stable manner.

### **Objectives**

This study aims to characterize antifungal susceptible profile of *C. parapsilosis* sensu stricto etiologic agent of human infections from two major Portuguese hospitals, Centro Hospitalar de Coimbra and Centro Hospitalar São João and uncover the molecular mechanisms underlying antifungal resistance.

### **Methods**

Clinical isolates (n=93) collected from distinct body sites/products were depicted regarding antifungal susceptibility profile to azoles (fluconazole, voriconazole, posaconazole) and echinocandins (caspofungin, micafungin, anidulafungin). The minimal inhibitory concentration (MIC) of each antifungal drug was determined according to the M27-A3 protocol of the Clinical and Laboratory Standard Institute. Gene expression profile of the resistant isolates was carried out by RT-PCR.

### **Conclusions**

Among *C. parapsilosis* isolates, 10% were resistant to fluconazole; 6% were resistant to voriconazole and 2% were resistant to posaconazole. The incidence of susceptible–dose-dependent found was 6% for FLC and 10% for VRC. No resistance was found for echinocandins. Azole resistance development was mainly associated with *MDR1* (encoding multidrug efflux pump) and *MRR1* (transcription factor that

regulates *MDR1* expression) genes overexpression. Our study showed that prevalence of azole *C. parapsilosis* resistant isolates has increased dramatically since the last survey carried out in 2009. This study was supported by FEDER, COMPETE and FCT (PTDC/DTP-EPI/1660/2012).

**FEMS-1494**

**Antimicrobial resistance**

## **IMPACT OF THE MUTATIONS IN DNA GYRASE GENES ON QUINOLONE RESISTANCE IN *CAMPYLOBACTER JEJUNI***

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### **Background**

*C. jejuni* has shown increasing resistance to quinolone. The resistance is mediated by a point mutation in the quinolone resistance-determining region (QRDR) of *gyrA* with the high frequency at position 86, followed by position 90. However, no direct evidence of the association between these mutations and the quinolone resistance has been shown to date.

### **Objectives**

To clarify the impact of an amino acid substitution at positions 86 or 90 in *C. jejuni* GyrA on DNA gyrase activity in the presence or absence of quinolones

### **Methods**

The recombinant DNA gyrases with amino acid substitutions at position 86, and 90 in GyrA were expressed in *Escherichia coli* and purified using Ni-NTA agarose column chromatography. Wild-type and mutant DNA gyrases were reconstituted *in vitro* by mixing recombinant GyrA and GyrB. Correlation between the amino acid substitution and quinolones resistance was assessed by quinolone-inhibited supercoiling assay.

### **Conclusions**

The quinolone-inhibited supercoiling assay demonstrated the important role of these amino acid substitutions in reduced sensitivity to quinolone with mark influence by amino acid substitution, especially Thr86Ile. This study confirmed the association of these mutations with the quinolone resistance in *C. jejuni* and demonstrated that sitafloxacin might be a candidate for the treatment of campylobacteriosis caused by ciprofloxacin-resistant *C. jejuni*.





**FEMS-1999**

**Antimicrobial resistance**

**CLASS 1 INTEGRON-INTEGRASE ACTIVITY AND BACTERIAL SOS RESPONSE  
IN A MOUSE MODEL**

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**Background**

The bacterial SOS response to DNA damages is involved in antibiotic resistance acquisition and dissemination. It can regulate expression of resistance genes (eg *qnrB*), or acquisition of resistance genes (eg expression of the integrase of integrons, *IntI*). Integrons are genetic elements that play a major role in the dissemination of antibiotic resistance in Gram-negative bacteria. *IntI* catalyses insertion and excision of resistance genes embedded within gene cassettes. Dissemination of resistance genes might also be correlated to the SOS response as shown for bacterial conjugation. Various stresses including antibiotics can induce the SOS response.

**Objectives**

Our aim was to estimate the level of the SOS response and of the *IntI* expression and activity *in vivo* in the mouse digestive tract.

**Methods**

Germ-free mice were inoculated intragastrically with a strain of *Escherichia coli* carrying two plasmids: one harbouring the *intI1* gene under the control of its own promoter, the second containing a gene cassette array. Efficiency of bacterial colonisation, plasmid stability and *IntI1* activity were estimated from mice feces. *intI1* and *sfiA* (SOS regulon) genes expression level was estimated by qPCR.

**Conclusions**

We observed a higher expression of the SOS response and of *IntI1* *in vivo* than *in vitro* and a 1.5 log increase of the *IntI1* excision frequency.

Our results indicate that the digestive tract is an environment where the SOS response is induced. Moreover, the higher expression and activity of integrase highlight the role of the gut in disseminating antibiotic resistance.

## FEMS-0889

### Antimicrobial resistance

#### A DIFFERENT ATP-BINDING CASSETTE (ABC) SYSTEM LIKELY ASSOCIATED WITH LINEZOLID RESISTANCE IN STAPHYLOCOCCUS HAEMOLYTICUS

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#### Background

Linezolid is an oxazolidinone antimicrobial agent for treating infections caused by multi-drug resistant Gram-positive cocci. Mutations in domain V of 23S rRNA, an adenosine modification catalyzed by Cfr and mutations in the ribosomal proteins L3, L4 and L22 are mechanisms that have emerged in linezolid-resistant isolates. We identified a clinical *Staphylococcus haemolyticus* strain (ShRL1) exhibiting a high level of minimum inhibitory concentrations (MIC, 64µg/mL) for linezolid, which did not show any of these mechanisms. Moreover, the ShRL1 strain shows genes encoding proteins of the ATP-binding cassette (ABC) superfamily which were not identified in its susceptible derivative ShSL2 (MIC, 1µg/mL) after 7 subcultures under no selective antibiotic pressure.

#### Objectives

To compare the whole genome sequencing of the ShRL1 and ShSL2 strains in order to determine a cause of linezolid resistance.

#### Methods

Genome sequencing was performed using the Ion Torrent<sup>®</sup> PGM platform (Life Technologies) kit400bp. The sequences were de novo assembled using Mira v4.0.2 and spades v3.1.1. Scaffold was obtained using CLC Genomics Workbench v7.5 and Geneious 8.0.5 with the genome reference *S. haemolyticus* JCSC1435. Lastly comparative analyses were performed using Mauve v3.2.

#### Conclusions

Rare studies have associated efflux-pump systems with linezolid decreased susceptibility. We propose a resistance mechanism other than target site modifications that may be responsible for elevated MIC for linezolid in *S. haemolyticus*. Further studies are necessary to support it. However, our data strongly suggest that the ATP-binding cassette (ABC) system, which share about 70% sequence homology to efflux proteins of *S. aureus*, has an important role in linezolid resistance in *S. haemolyticus*.

**FEMS-0596**

**Antimicrobial resistance**

## **THE ANTIBACTERIAL EFFICACY OF DIFFERENT MOUTHRINSES ON PERIODONTAL PATHOGENS**

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### **Background**

The widespread use of systemic antibiotics in medicine and dentistry becomes an increasingly serious problem by eliciting drug resistance and undesirable side effects. In dentistry, there are several antiseptic mouthrinses for topical use available, showing different mechanisms of action to inhibit dental pathogens, for instance those associated with periodontitis and peri-implantitis.

### **Objectives**

Aim of the study was to investigate the antimicrobial efficacy of eight antiseptic mouthrinses containing six different types of active ingredients for the use on planktonic periodontitis-associated pathogens.

### **Methods**

Six representative periodontitis/peri-implantitis associated pathogens were grown anaerobically for 24h. After suspension, the six-strain-cocktail was exposed to the following agents for 30 seconds:

1. NaCl (saline as negative control), 2. CHX (0.2%) (chlorhexidine), 3. CHX (0.06%), 4. CHX+CPC (CHX 0.05% + cetylpyridinium chloride 0.05%), 5. PH (polyhexanide 0.04%), 6. PH 0.1%+betaine, 7. Oct (octenidine dihydrochloride + phenoxyethanol), 8. FF (amine fluoride/stannous fluoride), and 9. EO (mouthwash containing essential oils). Microbial viability was monitored by both colony growth (CFU/ml) and fluorescence-based vitality. Six independent series of experiments were conducted. Statistical analysis of data was performed using mean-based 95% confidence intervals, ANOVA analysis and the Tukey test.

### **Conclusions**

The strongest antibacterial CFU reduction of 8.75 log<sub>10</sub> units could be shown for the agents 2, 4 and 7. Furthermore, strong colony growth reduction of 7.25-6.36 was

caused by the agents 6, 8 and 9. Moderate to low antibacterial activity was induced by the exposure to agents 3 and 5 ( $\log_{10}$  CFU reduction of 3.80-2.25). Bacterial vitality was strongly reduced by all antiseptic agents.

## **FEMS-2888**

### **Antimicrobial resistance**

#### **CHARACTERIZATION OF ESBL PRODUCING ESCHERICHIA COLI IN NORTHERN THAILAND**

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#### **Background**

The prevalence of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* has reached to 41-44 % of total *E. coli* infections in a tertiary center Maharaj Nakorn Chiang Mai Hospital (MNCMH) during past six years. To date, there is no report on distribution of ESBL genes and levels of resistance to third generation cephalosporins among *E. coli* clinical isolates from any hospital in Northern Thailand.

#### **Objectives**

To study the correlation between distribution of ESBL genes and levels of resistance to cefotaxime and ceftazidime.

#### **Methods**

435 *E. coli* strains collected from MNCMH during April to May, 2010 were screened for production of ESBL and AmpC BL. MICs of cefotaxime and ceftazidime were determined in ESBL producers. ESBL and AmpC BL genes were detected using multiplex PCR and nucleotide sequencing.

#### **Conclusions**

Of 182 ESBL producers, 25 strains also produced AmpC BL. The MIC<sub>50</sub>/MIC<sub>90</sub> of CTX and CAZ were 128/512 and 16/128 µg/ml, respectively. ESBL genes detected were *bla*<sub>TEM</sub> (82.9%), *bla*<sub>CTX-M</sub> (80.7% ; *bla*<sub>CTX-M-group 1</sub> and *bla*<sub>CTX-M-group 9</sub>), and *bla*<sub>SHV</sub> (11%). *bla*<sub>CTX-M-15</sub> and *bla*<sub>CTX-M-55</sub> predominated among *bla*<sub>CTX-M-group 1</sub> whereas *bla*<sub>CMY2</sub> among AmpC BL producers. Most strains carried multiple beta-lactamase genes. The predominant genotypes were *bla*<sub>TEM</sub> + *bla*<sub>CTX-M-group 1</sub> and *bla*<sub>TEM</sub> + *bla*<sub>CTX-M-group 9</sub>. Levels of resistance to cefotaxime and ceftazidime depended on type of beta-lactamase gene, not the accumulating number of genes. The presence of *bla*<sub>CTX-M-group 1</sub> was associated with resistance to both cefotaxime and ceftazidime. However, the

presence of *bla*<sub>CTX-M-group 9</sub> was associated with more resistance to cefotaxime and more sensitivity to ceftazidime.

## **FEMS-2335**

### **Antimicrobial resistance**

## **FITNESS STUDIES OF PZA RESISTANT MYCOBACTERIUM TUBERCULOSIS STRAINS**

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### **Background**

Pyrazinamide (PZA) is the only first line tuberculosis (TB) drugs to have significant activity against dormant TB. PZA resistance in *Mycobacterium tuberculosis* is most frequently conferred by mutations in pyrazinamidase (PZase), the enzyme required to activate PZA. These resistance mutations are sometimes stated to have no fitness cost, but in fact laboratory evidence seems absent for either a fitness cost or lack thereof.

PZase is non-essential during active growth, but its transcription is upregulated during oxygen and nutrient limitation. The role of PZase in NAD salvaging supports requirement of PZase during dormancy, and thus PZA resistant strains without functional PZase may have a measurably decreased fitness. Epidemiological data showing only limited spread of PZA resistant strains also suggests a lower fitness.

### **Objectives**

Objective of this study is to determine the fitness of PZA resistant TB strains in a dormancy model.

### **Methods**

Isogenic PZA susceptible and resistant strains of *M. tuberculosis* are cultured in the Wayne hypoxic dormancy model in non-selective media, and viability investigated over time.

### **Conclusions**

In the first 2 months of dormancy model culture, proportions of both the PZA resistant and susceptible bacteria remained viable as determined by live/dead staining and culture. Auramine/NileRed stainings demonstrate decreasing proportions of acid-fast bacilli and increasing proportions of lipid inclusion over time in susceptible and resistant strains.



No measurable difference in fitness was observed between the strains during the first two months, viability will be further assessed for additional time points to assess the long term survival of these dormant cultures.

**FEMS-3069**

**Antimicrobial resistance**

## **ANTIBACTERIAL ACTIVITY AND MECHANISM OF ACTION OF EUGENOL AGAINST MULTI-DRUG RESISTANT PATHOGENIC BACTERIA**

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### **Background**

Antibiotic resistance in clinical isolates is an increasing life-threatening global threat. Newer approaches and drugs are required to control these organisms in the current scenario. The *in vitro* antibacterial activity and mechanism of eugenol, a natural phenylpropene was studied against multi-drug resistant clinical isolates.

### **Objectives**

The present study was conducted to evaluate the antibacterial property of eugenol against multi-drug resistant clinical bacterial pathogens. Further, minimum inhibitory concentration and bactericidal concentration of eugenol along with mechanistic action was determined.

### **Methods**

Antimicrobial minimum inhibitory concentration and bactericidal concentration experiments with eugenol was measured against a range of Gram positive and Gram negative bacteria isolated from clinical specimens using Resazurin micro-titre assay. Analysis of killing kinetics of eugenol was performed against two strains (*Escherichia coli* and *Staphylococcus aureus*). The effect of divalent salts on the activity of eugenol was determined. Bioluminescence method was used to determine the effect of eugenol on the intracellular pool of bacterial ATP. Membrane depolarization studies were conducted to study the cell wall permeabilization. The morphological changes in bacterial membrane due to the activity of eugenol were investigated using scanning and transmission electron microscopy studies.

### **Conclusions**

The results provide additional experimental data that are consistent with the hypothesis that eugenol represents a new class of antibacterial agents for treating multi-drug resistant pathogenic bacteria and acts *via* different mechanism from conventional antibiotics. The results suggest that antibacterial activity is due to

interaction of eugenol on bacterial cell membrane. The results also revealed that cell wall and membrane of treated bacteria were significantly damaged.

## **FEMS-2282**

### **Antimicrobial resistance**

#### **PREVALENCE AND CHARACTERIZATION OF BLA CTX-M, BLA TEM AND BLA SHV FROM ESBL PRODUCING ORGANISMS FROM HOSPITALS IN LAGOS, NIGERIA.**

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### **Background**

The increasingly prevalent of Extended-Spectrum  $\beta$ -Lactamase (ESBL) among clinical isolates are becoming wide spread globally. Most reports on ESBL from Nigeria have always rested on their phenotypic statues with just a few with information on their molecular types.

### **Objectives**

We therefore set out to to characterize the Beta-lactamases in multidrug resistant clinical isolates of *Enterobacteriaceae* by molecular techniques.

### **Methods**

One hundred and sixteen non-duplicate clinical isolates from 3 hospitals including a Teaching hospital were screened for ESBL production, 42 of them were phenotypically confirmed as ESBL producers. Molecular analysis by PCR and Pulsed Gel Electrophoresis used in the detection of TEM, SHV and CTX-M ESBL genes revealed 100% prevalence for CTX-M, 57.1% TEM and 64.3% SHV. *Klebsiella pneumoniae* and *E. coli* respectively had the most frequency of ESBL genes with *Serratia rubidae* with the lowest frequency of occurrence. All ESBL producers showed resistance to at least 2 or more classes of antibiotics and varied resistance to meropenem (carbapenem).

### **Conclusions**

Despite several researches and reports on ESBL in Nigeria, there is still no known awareness for its routine detection in the private and public hospital laboratories. It

seems as though the fact is simply ignored. This study has brought to light the extent of the spread of these organisms in Lagos and call for stringent policies to be put in place to reduce antibiotic misuse and abuse.

## **FEMS-1854**

### **Antimicrobial resistance**

#### **RETROSPECTIVE DETECTION OF THE HIDDEN SPREAD OF OXA-48-PRODUCING KLEBSIELLA PNEUMONIAE IN A FRENCH TEACHING HOSPITAL**

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### **Background**

In our hospital, an outbreak of OXA-48-producing *Klebsiella pneumoniae* (OXA48KP) was identified in the end of 2013. For these strains, the Vitek2 System (V2S) categorised the ertapenem intermediate or resistant (I/R), whereas it could have been categorised susceptible by E-test (E-test is systematically performed if ertapenem is categorised I/R with the V2S). Before the outbreak, no screening for carbapenemases was performed for isolates identified as susceptible to ertapenem by E-tests.

### **Objectives**

The objective of this study was to determine the presence of OXA48KP before the moment of the outbreak detection.

### **Methods**

From January 2010 to the end of 2013, all *K. pneumoniae* isolates for which ertapenem was tested by E-test were selected. These isolates were then tested for the presence of carbapenemase with the KPC, MBL and OXA-48 confirm Kit (ROSCO Diagnostica, Taastrup, Denmark). The isolates with an inhibition diameter around temocillin <12 mm were then tested for OXA-48 by PCR and compared by PFGE.

### **Conclusions**

Among the 21 isolates included, 3 were identified as OXA48KP. PFGE analysis demonstrated that two of them were closely-related to the outbreak isolates and one was not related. They had been initially considered susceptible by E-test (MIC from 0.25 to 0.5 mg/L) and had been identified from samples collected within the 6 months preceding outbreak detection. Therefore, there was probably a hidden spread of the outbreak before triggering the alert at the end of 2013. In addition, these results show the high sensitivity of the V2S for carbapenemase detection.



## **FEMS-1010**

### **Antimicrobial resistance**

#### **CHARACTERIZATION OF THE RESISTANCE MECHANISM TO DITYROMYCIN OF THE STREPTOMYCES PRODUCER STRAIN.**

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#### **Background**

Dityromycin is a peptide antibiotic produced by *Streptomyces* sp. strain AM-2504<sup>1</sup> with the structure highly related to that of GE82832<sup>2</sup>. The crystal structure of Dityromycin - 70S ribosome complex revealed that the binding involves conserved amino acid residues of ribosomal protein S12<sup>3</sup>. Dityromycin inhibits EF-G activity by disrupting critical contacts between EF-G and S12 that are required to stabilize the post-translocational conformation of EF-G, thereby preventing the ribosome-EF-G complex from entering a productive arrangement for translocation<sup>3</sup>.

#### **Objectives**

The aim of this work was to identify the mechanism of resistance to Dityromycin of the producer strain *Streptomyces* sp. AM-2504.

#### **Methods**

Cell-free *in vitro* translation tests, genome walking and direct sequencing of the *rpsL* gene were used to characterize the mechanism of resistance of this antibiotic.

#### **Conclusions**

The results obtained in this work indicate that the resistance of the producer strain to Dityromycin is due to a modification of its ribosomes. In particular, three amino acids changing mutations were found in an highly conserved region of the protein S12 corresponding to the binding site of the antibiotic. These mutations cause a substantial loss of affinity of Dityromycin for the 30S ribosomal subunit thus conferring resistance to the producer strain. Results of this study contribute to the knowledge of the molecular determinants developed and successfully implemented by the antibiotic production/resistance machinery.

#### **References**

- 1) Teshima et al. (1988). *Tetrahedron Lett.* 29: 1963-1966.
- 2) Brandi et al. (2012). *FEBS Lett.* 586: 3373-3378.



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**FEMS-2599**

**Antimicrobial resistance**

**HAS THE USE OF FLUOROQUINOLONES CONTRIBUTED TO THE WIDESPREAD DISSEMINATION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AND EXTENDED-SPECTRUM B-LACTAMASE-PRODUCING KLEBSIELLA PNEUMONIAE IN THE HEALTHCARE SETTING?**

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**Background**

We recently demonstrated that fitness cost associated with resistance to fluoroquinolones is diverse across clones of both methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Klebsiella pneumoniae* in adult hospital wards. We observed that major clone MRSA and ESBL-producing *K. pneumoniae* strains with high-level resistance to fluoroquinolones retain their fitness while the vitality of other *S. aureus* and *K. pneumoniae* isolates become more compromised during the development of resistance to these antibiotics. Our observation with MRSA was subsequently confirmed by British scientists. In addition our findings may account for the replacement of SHV type ESBL genes by CTX-M-15 in *K. pneumoniae* reported during the last decade.

**Objectives**

Investigation of whether or not this mechanism - the selection of the major clones of MRSA and ESBL--producing *K. pneumoniae* by fluoroquinolones - could have contributed to the widespread dissemination of both pathogens.

**Methods**

Review of related literature.

**Conclusions**

An overwhelming part of the related literature supports the assumption.

Our findings suggest that a more judicious use of fluoroquinolones could significantly decrease the rates for both MRSA and ESBL-producing *K. pneumoniae* in the adult healthcare setting.

## FEMS-2925

### Antimicrobial resistance

#### WHICH ANTIBIOTIC SHOULD BE PRESCRIBED FOR ERADICATION OF KLEBSIELLA PNEUMONIAE NOSOCOMIAL INFECTIONS IN ILAM HOSPITALS? AMOXICILLIN OR AMIKACIN?

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#### Background

The relax prescription of antibiotics in Iran made an evolution in bacterial populations. Antibiotic resistance strains specially noscomial strains cause too issues for treatment of bacteria responsible for nosocomial infections. *Klebsiella pneumoniae* is also known as a hard to treat bacterium in Ilam hospitals and the rate of resistance is different from antibiotic to antibiotic.

#### Objectives

Therefore, the current study aims to evaluate the prevalence of different antibiotics for treatment of *K. pneumoniae* clinical isolates and open new insight for antibiotic suspension or prescription.

#### Methods

48 *K. pneumoniae* clinical isolates were identified as standard procedures during March 2013 to March 2014 in Ilam hospitals. The isolates were collected from in and out patients with urinary tract infections. The isolates were then subjected to antibiotic susceptibility testing via disk diffusion method as CLSI guideline. The antibiotic resistance pattern and significant difference among *K. pneumoniae* and different antibiotics were calculated by spss 16 via chi-square.

#### Conclusions

The antibiotic susceptibility pattern demonstrated 39.6%, 27.1%, 27.1%, 16.7%, 39.6%, 37.5%, 10.4%, 54.2%, 8.3%, 75%, 27.1%, 50%, and 97.9% resistance to gentamicin, ciprofloxacin, ceftazidime, piperacillin, aztreonam, chloramphenicol, nitrofurantoin, cephalotin, amikacin, cefuroxime, tetracycline, cephazolin, and amoxicillin, respectively. The findings demonstrated high resistance in *K. pneumoniae* clinical strains. This study recommended the new policy with prescription of amikacin and non-prescription of amoxicillin as a dominant susceptible and resistance antibiotic in *K. pneumoniae* clinical isolates.

FEMS-2127

Antimicrobial resistance

## ADES MUTATIONS IN MDR-ACINETOBACTER BAUMANNII CLINICAL ISOLATES WITH TIGECYCLINE REDUCED SUSCEPTIBILITY FROM CAIRO, EGYPT.

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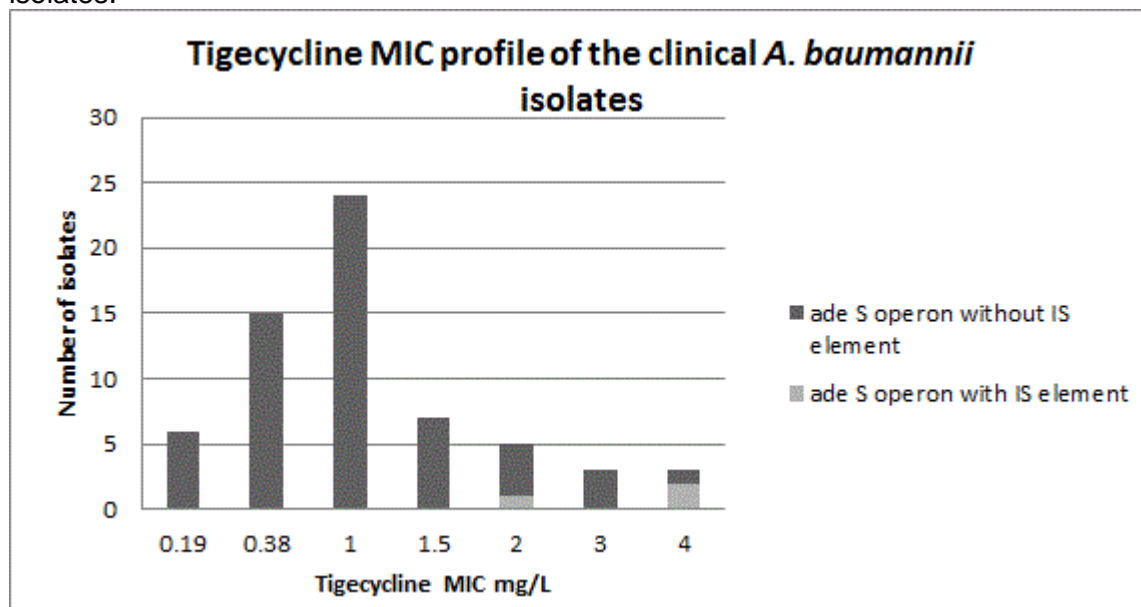
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### Background

Infections caused by multidrug resistant (MDR) *Acinetobacter baumannii* (*A. baumannii*) especially in intensive care units have limited therapeutic options, one of them is Tigecycline. Overexpression of the adeABC efflux pump may be caused either by the IS*Aba*-1 insertion or by specific point mutations in *adeR* and *adeS*, therefore plays a major role in conferring MDR- *A. baumannii*.

### Objectives

We aimed in our study to monitor the TGC susceptibility and to study the role of IS*Aba*-1 and the *adeS* regulator within the AdeABC efflux pump among MDR- *A. baumannii* clinical isolates.



### Methods

Over 18 months from April 2012 to September 2013, 63 MDR-*A. baumannii* identified by detection of OXA-51 like gene were isolated from ICU patients. TGC MIC was determined by E test and confirmed by broth microdilution. PCR analysis of *adeR*, *adeS*, *adeB*, and IS*Aba*1 genes were done with further sequencing of *adeS* gene.

### Conclusions

Reduced susceptibility to TGC (MIC: 3-4ml/L) was noticed in 6/63 (9.5 %) MDR- *A.*

*baumannii* isolates, insertion sequence IS*Aba*-1 was detected in 3 isolates , two of them showed amino acid substitutions in the *adeS* operon by sequencing. IS*Aba*-1 may play a role in conferring reduced TGC susceptibility in MDR- *A. baumannii* in association with amino acids mutations in the *adeS* gene

**FEMS-1847**

**Antimicrobial resistance**

**MOLECULAR CHARACTERIZATION AND EPIDEMIOLOGY OF CEFOTAXIME RESISTANCE AMONG ENTEROBACTERIACEAE LACKING INDUCIBLE CHROMOSOMAL AMPC GENES FROM HOSPITALIZED AND NON-HOSPITALIZED PATIENTS IN ALGERIA**

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**Background**

Plasmid-mediated AmpC  $\beta$ -lactamases have emerged and are being reported worldwide with varying prevalence rates. In Algeria, only few reports on plasmid-encoded AmpC in *Enterobacteriaceae* strains recovered from hospital settings were published.

**Objectives**

The aim of this study was to investigate the prevalence and molecular epidemiology of cefotaxime resistance among *Enterobacteriaceae* isolates recovered from hospitalized and non-hospitalized patients in Bejaia locality (Algeria). The association of pAmpC with ESBL and plasmid-mediated quinolone resistance determinant was also studied.

**Methods**

In this study, 922 consecutive non-duplicate clinical isolates of *Enterobacteriaceae* obtained from hospitalized and non-hospitalized patients at Bejaia, Algeria, were analyzed for AmpC type  $\beta$ -lactamases production. The *ampC* genes and their genetic environment were characterized using PCR and sequencing. Plasmid incompatibility groups were determined using PCR-based replicon typing. Phylogenetic grouping and Multilocus sequence typing (MLST) were determined for molecular typing of the plasmid encoded AmpC (pAmpC)-producing isolates.

**Conclusions**

Of the isolates, 15 were identified as AmpC producers including 14 CMY-4-producing isolates and one DHA-1-producing *K. pneumoniae*. All AmpC-producing isolates co-expressed the broad-spectrum TEM-1  $\beta$ -lactamase and three of them co-produced CTX-M and/or SHV-12 ESBL. Phylogenetic grouping and virulence genotyping of the

*E. coli* isolates revealed that most of them belonged to groups D and B1. MLST analysis of *K. pneumoniae* isolates identified four different STs with two new sequences: ST1617 and ST1618. Plasmid replicon typing indicates that *bla*<sub>CMY-4</sub> gene was located on broad host range A/C plasmid, while LVPK replicon was associated with *bla*<sub>DHA-1</sub>. All isolates carrying *bla*<sub>CMY-4</sub>, displayed the transposon-like structures ISEcp1/ΔISEcp1-*bla*<sub>CMY</sub>-*blc*-*sugE*.

## FEMS-1402

### Antimicrobial resistance

#### ENTEROCOCCAL COLONIZATION AND INFECTION IN LUNG TRANSPLANT PATIENTS WITH VENTILATOR ASSOCIATED PNEUMONIA: QUANTITATIVE CULTURE, DRUG SUSCEPTIBILITY TESTING AND PFGE PROFILING

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#### Background

Vancomycin-resistant enterococci (VRE) are among the most common antibiotic-resistant nosocomial pathogens in health care in general and in intensive care units (ICUs), in particular.

#### Objectives

The main purpose of this study is to distinguish between lower respiratory tract (LRT) colonization and infection due to enterococci using semi-quantitative cultures.

#### Methods

Sixty-eight lower respiratory tract (LRT) specimens were collected from consecutive adult patients who underwent Lung transplantation and were developed ventilator associated pneumonia (VAP) from three teaching hospitals in Tehran, Iran, during December 2009 – September 2012. A semi-quantitative threshold of  $>10^4$  cfu/ml was considered to be diagnostic for Enterococcal-VAP. Antibacterial resistance patterns of isolates to 13 antimicrobial agents were analyzed according to EUCAST. Pulsed Field Gel Electrophoresis (PFGE) was performed as described previously (Feizabadi, M.M. *et al.* J Microbiol Methods 2011; 84:144 –146).

#### Conclusions

Twenty-three and four isolates of *E. faecalis* and *E. faecium* showed  $>10^4$  cfu/ml which corresponds to infection. Linezolid, teicoplanin and vancomycin were found to be the most effective drugs.

The results of MIC for vancomycin and teicoplanin are shown in the table. Seven and two different DNA banding patterns were identified among *E. faecalis* and *E. faecium* isolates, respectively.

Infection of LRT with enterococci seems to become serious and needs to be reconsidered as a real problem rather than colonization. In such cases clones of VRE is associated with high mortality.

	Number of isolates	MIC of vancomycin 64≥mg/mL	MIC of vancomycin 256≥mg/mL	MIC of teicoplanin



				256≥mg/mL
<i>E. faecalis</i>	26	7	5	4
<i>E. faecium</i>	4	4	4	4

## **FEMS-2131**

### **Antimicrobial resistance**

#### **EVALUATION OF MULTIPLEX TANDEM REAL TIME PCR KIT FOR RAPID IDENTIFICATION OF GRAM-POSITIVE COCCI FROM BLOOD CULTURES AND DETECTION OF MECA AND VAN GENES**

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#### **Background**

Currently available automated blood culture systems have significantly reduced bacteria detection time. However; so as to get optimum treatment, more rapid identification systems are required.

#### **Objectives**

Gram Positive 12 Kit (Aus Diagnostics, Australia) makes it possible to identify common Gram positive bacteria (*Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp.) and to detect common antibiotic resistant genes (*mecA*, *vanA*, *vanB*). The aim of this study was to rapidly identify and to assess the performance of this test for sepsis agent Gram positive bacteria and resistance genes.

#### **Methods**

54 patients having positive blood cultures and Gram-positive cocci in Gram staining were included in the study. After the DNA isolation, identification and resistance genes were determined by using Easy Plex pipetting robot, and then Rotor GeneQ (Qiagen, Switzerland) real-time PCR device for pre- PCR and PCR set up phases with this multiplex tandem real time PCR kit. Results were compared to the results obtained from PHOENIX (BectonDickinson, USA) and Vitek 2 Compact (bioMérieux, France) automated systems.

#### **Conclusions**

42 results were completely as same as the results from PHOENIX and Vitek 2 Compact automated systems. Additionally; 12 polymicrobial infections which could not be detected by automated systems were successfully detected.

In conclusion; the kit which was evaluated proved to be both rapid (approximately 2,5 hours) and reliable in determining common Gram positive sepsis pathogens and resistance genes. According to our results, it could be concluded that the test could easily be used in patients who are suspected of sepsis in routine diagnosis.



## **FEMS-2734**

### **Antimicrobial resistance**

#### **RAPID IDENTIFICATION OF GRAM-NEGATIVE PATHOGENS FROM BLOOD CULTURES AND DETECTION OF RESISTANCE GENES**

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#### **Background**

Currently available automated blood culture systems have significantly reduced bacteria detection time. However; so as to get optimum treatment, more rapid identification systems are required.

#### **Objectives**

Gram negative 12 Kit (Aus Diagnostics, Australia) makes it possible to identify common Gram negative bacteria (Enterobacteriaceae, Pseudomonas spp., Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae) and to detect common antibiotic resistant genes. The aim of this study was to rapidly identify and to assess the performance of this test for sepsis agent Gram negative bacteria and resistance genes.

#### **Methods**

78 patients having positive blood cultures and Gram-negative bacilli in Gram staining were included in the study. After the DNA isolation, identification and resistance genes were determined by using Easy Plex pipetting robot, and then Rotor GeneQ (Qiagen, Switzerland) real-time PCR device for pre- PCR and PCR set up phases with this multiplex tandem real time PCR kit. Results were compared to the results obtained from PHOENIX (Becton Dickinson, USA) and Vitek 2 Compact (bioMérieux, France) automated systems.

#### **Conclusions**

All of the pathogens were successfully identified by using this test. Oxa-23 gene, aadA1 gene, pan-vim genes, aadB gene were found in 5, 15, 3, 2 samples; respectively.

In conclusion; the kit which was evaluated proved to be both rapid (approximately 2,5 hours) and reliable in determining common Gram negative sepsis pathogens and resistance genes. According to our results, it could be concluded that the test could easily be used in patients who are suspected of sepsis in routine diagnosis.



**FEMS-1689**

**Antimicrobial resistance**

## **ANTIFUNGAL SUSCEPTIBILITY TESTING OF CLINICAL DERMATOPHYTE ISOLATES FROM IRAN**

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### **Background**

Dermatophytosis is one of the most common fungal infections in humans. In vitro antifungal susceptibility testing is considered as a powerful method to seek the drug of choice among the available anti-dermatophyte agents as well as to optimize the therapy against dermatophytosis.

### **Objectives**

we aimed the antifungal susceptibility of isolated dermatophytes from Iran against some common antifungal agents for the treatment of dermatophytosis.

### **Methods**

The isolates were identified by traditional macroscopic and microscopic findings as well as PCR-RFLP. A total of 316 clinical isolates from patients with dermatophytosis including *Trichophyton interdigitale* ( $n = 156$ ), *T. rubrum* ( $n = 60$ ), *Epidermophyton floccosum* ( $n = 42$ ), *Microsporum canis* ( $n = 29$ ), *Arthroderma benhamiae* ( $n = 17$ ) and *T. tonsurans* ( $n = 12$ ) were tested for antifungal activities against terbinafine, itraconazole, griseofulvin and fluconazole by broth microdilution technique according to the recommendation of the Clinical and Laboratory Standards Institute (CLSI) M38-A2 document.

### **Conclusions**

The MIC ranges were as follows: terbinafine, 0.002 to 0.25 µg/mL; itraconazole, 0.004 to 0.5 µg/mL; griseofulvin, 0.125 to 8 µg/mL and fluconazole 4 to 128 µg/mL. Terbinafine and fluconazole had the highest and lowest activity against dermatophytes isolates, respectively. *Epidermophyton floccosum*, *Microsporum canis* and *Trichophyton tonsurans* were the most susceptible species to terbinafine. Terbinafine had the lowest activity against *Arthroderma benhamiae*. *E. floccosum* showed the highest susceptibility to itraconazole. *T. rubrum* had the lowest

susceptibility to itraconazole. According to our data we profoundly propose to physicians to exclude fluconazole for treatment of dermatophytosis.

## FEMS-2959

### Antimicrobial resistance

#### SRL PATHOGENICITY ISLAND IS ASSOCIATED TO MULTIDRUG RESISTANCE IN SHIGELLA STRAINS ISOLATED FROM CHILE

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#### Background

*Shigella* spp., the etiologic agent of shigellosis, becomes a major problem of public health due to the increasing multidrug-resistance to antibiotic (MDR). In this context, the *Shigella* resistance locus (SRL) pathogenicity island (PAI) mediates resistance to streptomycin, ampicillin, chloramphenicol, and tetracycline, contributing to MDR phenotype.

#### Objectives

To characterize the presence and genetic organization of SRL PAI in Chilean *Shigella* spp. strains.

#### Methods

We selected 135 strains of *S. sonnei* and *S. flexneri*. We determined susceptibility to antibiotics using agar diffusion method, and detected seven specific SRL markers (*orf9*, *orf58*, *oxa-1*, *tet*, *cat*, and its sites of insertion in the chromosome) by PCR. Also, we assessed the presence of integrons. Then, we selected representative strains and analyzed them by Tilling-PCR for the complete island organization.

#### Conclusions

Our results indicate that most of these strains are MDR strains to  $\geq 3$  antibiotic class ( $> 78\%$ ), all of them had the SRL PAI. Besides, a high rate of the isolated was positive for class 1 integron (80%), which is concordant with the presence of the SRL PAI and negative for integron class 2.

Meanwhile, tilling-PCR for SRL PAI and sequencing indicated that in the 5' end there are four variants with different insertions. Only one insertion was found in the 3' end, compared to the reference strain, YSH6000. In conclusion, this work points out the diversity of variants of the SRL PAI in the Chilean *Shigella* spp strains, and its huge contribution to the MDR phenotype.

Supported by FONDECYT-1130394



**FEMS-0636**

**Antimicrobial resistance**

## **MOLECULAR EPIDEMIOLOGY OF PLASMIDS MEDIATING OXA-48 CARBAPENEMASE DISSEMINATION**

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### **Background**

OXA-48 carbapenemase has become of major significance as a result of the progressive identification of nosocomial *Klebsiella pneumoniae* producing this enzyme. It has been reported mostly in Mediterranean countries, although it is increasingly detected in Northern Europe. The recent spread of a clonal OXA-48-producing *K. pneumoniae* as well as other *Enterobacteriaceae* in a Dutch hospital has resulted in one of the largest OXA-48 related outbreaks in Europe so far<sup>1</sup>.

### **Objectives**

The OXA-48 gene has been associated with 60- to 70-kb plasmids. Our aim was to perform a large-scale study using a next-generation sequencing approach to better understand the molecular epidemiology of plasmids carrying *bla*<sub>OXA-48</sub>.

### **Methods**

A total of 89 OXA-48-producing bacteria isolated from the Dutch hospital outbreak<sup>1</sup> as well as from other locations in the Netherlands and endemic countries were selected. Plasmids were sequenced using the Illumina Miseq platform, and read sets were assembled *de novo* using Spades. Plasmid sequences were annotated with Prokka, and Samtools was used to identify SNPs and INDELs.

### **Conclusions**

Sequencing data revealed 3 plasmid clusters of ca. 62kb, 30kb, and 13kb. All the plasmids shared an identical common region including the Tn 1999 where *bla*<sub>OXA-48</sub> is embedded, suggesting the presence of a highly modular plasmid ancestor. No link was found between plasmid clusters and origin of the strains. However, our results show several cases of plasmid transfer between bacterial species in the same patient over time, which represents a major driver for plasmid maintenance and spread despite control of the outbreak.

## References

- <sup>1</sup>. **Euro Surveill.** 2014;19(9).

**FEMS-2054**

**Antimicrobial resistance**

## **ANTIMICROBIAL ACTIVITY OF CHLORELLA VULGARIS EXTRACTS AGAINST FOODBORNE PATHOGENIC BACTERIA**

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### **Background**

Foodborne bacterial pathogens cause a major public health problem. In the last few decades, increased resistance of bacterial strains to antibiotics lead to search alternative effective compound against food borne pathogens.

### **Objectives**

In this study was aimed at evaluating the antimicrobial activity of different extracts from *Chlorella vulgaris* on food borne pathogens. Systematic screening of them may result in the discovery of novel effective compounds.

### **Methods**

*Chlorella vulgaris* (UTEX 2714) was grown in axenic cultures of 3N-Basal Bold Medium. Chloroform, methanol, ethanol, diethyl ether, acetone and ethyl acetate extracts were extracted directly from 5 mg dried *C.vulgaris* biomass. *Staphylococcus aureus*, *Listeria grayii*, *L.monocytogenes*, *L. innocua*, *Enterococcus faecalis* and *E. faecium* strains was isolated from cheese, minced meat and poultry product and identified by molecular techniques. Disc diffusion methods were used to determine the antimicrobial activity of *C. vulgaris* extracts on the foodborne pathogens. Results compared with streptomycin and amikacin results which was used as positive control.

### **Conclusions**

*C.vulgaris* extracts possessed potential antibacterial activities against foodborne gram positive bacterial strains with inhibition zones ranged for *S.aureus*, *E. faecalis* and *E.faecium* 7-14 mm, *L.monocytogenes* 7-12 mm, *L.grayi* 7-14 mm, *L.innocua* 7-15 mm, Thus, it could be suggested that the *C.vulgaris* is useful biosystem for production of new antimicrobial compound that has an effect against gram positive bacteria.

**FEMS-1576**

**Antimicrobial resistance**

## **THE GENOMES OF CARBAPENEM-RESISTANT PATHOGENS OF FIVE DIFFERENT SPECIES ISOLATED AT A SWEDISH HOSPITAL**

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### **Background**

Carbapenemases are able to hydrolyze carbapenems and, in several instances, almost all other  $\beta$ -lactam antibiotics, making these enzymes a great threat to our ability to treat refractory bacterial infections.

### **Objectives**

In this study, we aimed to determine the genetic context of carbapenemase genes and any additional acquired resistance genes in clinical isolates. We also wanted to study the potential of using whole genome sequencing (WGS) in predicting the antibiotic resistance profiles of multi-resistant isolates.

### **Methods**

Six clinical carbapenemase-positive isolates of five different species (*Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*) were selected at the Sahlgrenska university hospital in Gothenburg, Sweden. WGS of selected isolates resulted in draft assemblies that were annotated for mobile antibiotic resistance genes and chromosomal mutations in antibiotic target genes.

### **Conclusions**

We found that several different resistance genes and mutations had been acquired by the isolates. The detected carbapenemase genes included variants *bla*<sub>NDM</sub>, *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-48</sub>, and *bla*<sub>OXA-51</sub>, and some isolates carried multiple variants. From the acquired resistance mechanisms and the intrinsic resistance profile of each species, we could predict a susceptibility profile. The phenotypic resistance profile was determined by measuring the MIC for 37 different antibiotics belonging to all major classes, using E-tests. The results showed good cohesion between the predicted phenotype and measured resistance profile. Our results suggest that WGS can be used to predict the resistance phenotype of carbapenem-resistant isolates. The analysis also underlines the importance of using a comprehensive and curated database of resistance factors for accurate identification the resistance genotype.

## **FEMS-0653**

### **Antimicrobial resistance**

#### **SOS INDUCTION OF RCR-FAMILY TRANSPOSASES GENES**

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### **Background**

Our work focused on ISCR, a family of insertions sequences encoding RCR transposases thought to catalyze the a rolling-circle (RC) mechanism transposition of their IS elements. The prevailing ISCR, termed ISCR1, are associated to variable combinations of antibiotics resistance genes. The recent discovery of free forms of ISCR1 elements suggests their contribution to the dissemination of these genes.

### **Objectives**

Nothing is known about the expression of the RCR1-encoding gene *rcr1*. This gene displays a conserved s<sup>70</sup> promoter and a putative binding box (operator) for the LexA protein, the regulator of the SOS response. We therefore investigated the SOS induction of the expression of *rcr1*.

### **Methods**

Translational fusions *Prcr1-lacZ* with a wild type or a mutated LexA box were constructed. The activity of the *Prcr1* promoter was estimated using b-galactosidase assays performed in an *E.coli*  $\Delta lacZ$  strain or its derivative  $\Delta lexA$ , after treatment with an SOS-inducer or not.

### **Conclusions**

An activity from *Prcr1-lacZ* could be detected either in the  $\Delta lexA$  strain or upon exposure to mitomycin C unless in strains deficient for the SOS response ( $\Delta recA$ ). RNA techniques allowed us to map the *Prcr1* promoter that was also supported by mutation of the deduced -10 element.

Bioinformatics analyses suggested LexA could regulate more RCR transposases genes and we confirmed it with a recently identified transposase.

Our results highlight a SOS induction of *rcr* genes, suggesting that the mobility of their cognate mobile elements could be triggered upon exposure to some antibiotics or single-strand generating horizontal gene transfer events.

**FEMS-3095**

**Antimicrobial resistance**

**EPIDEMIOLOGIC CHARACTERIZATION OF NDM-1-PRODUCING ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE ISOLATES IN TURKISH HOSPITALS**

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**Background**

Enterobacteriaceae isolates producing New Delhi metallo-beta-lactamase-1 (NDM-1) are considered as a major health threat. We investigated the epidemiology of NDM-1-producing isolates among carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates submitted to a central laboratory from various Turkish hospitals.

**Objectives**

NDM-1 is a class B metallo-beta-lactamase conferring high-level resistance to beta-lactam antimicrobials including carbapenems and is transferable through plasmids. *bla*<sub>NDM-1</sub> positive Enterobacteriaceae are being reported from Turkish hospitals but the epidemiological characterization of these isolates is lacking. This study investigates the prevalence and clonal relationship of *bla*<sub>NDM-1</sub> positive isolates among carbapenem-resistant *E. coli* and *K. pneumoniae* collected from six major Turkish cities (Istanbul, Ankara, Bursa, Adana, Eskisehir, Kocaeli) between September 2011-March 2014.

**Methods**

We detected 143 carbapenem-resistant isolates (*E. coli*, n=28; *K. pneumoniae*, n=115) and a multiplex PCR assay targeting *bla*<sub>OXA-48</sub>, *bla*<sub>NDM-1</sub>, *bla*<sub>IMP</sub>, *bla*<sub>VIM</sub> and *bla*<sub>KPC</sub> genes was performed for these isolates. *bla*<sub>NDM-1</sub> was identified in 24 (16.7%) isolates (*E. coli*, n=4; *K. pneumoniae*, n=20). Pulsed-field gel electrophoresis (PFGE) of Xba-1-digested genomic DNA was used to assess the clonal relationship among *bla*<sub>NDM-1</sub> positive isolates which revealed 3 and 8 distinct pulsotypes among *E. coli* and *K. pneumoniae* isolates, respectively (Figure 1).

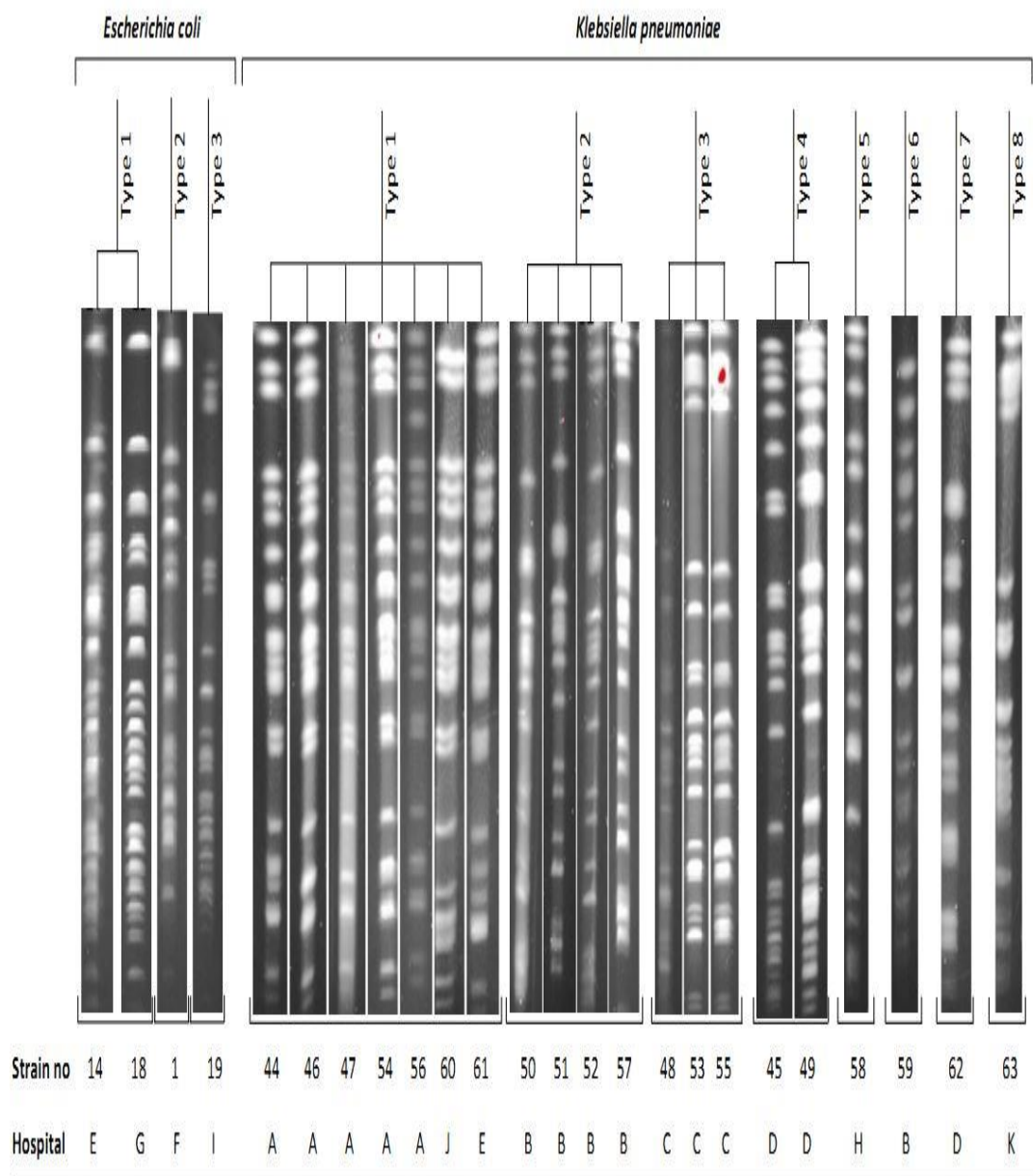


Figure 1. Pulsed-field gel electrophoresis patterns of *bla*<sub>NDM-1</sub> positive *Escherichia coli* and *Klebsiella pneumoniae* isolates

## Conclusions

*bla*<sub>NDM-1</sub> was detected in 14.3% and 17.4% of carbapenem-resistant *E. coli* and *K. pneumoniae* isolates, respectively. The different patterns obtained by the PFGE showed that the infections with NDM-1-producing *K. pneumoniae* are due to intra-institutional spread rather than transfer between institutions.

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## FEMS-0732

### Antimicrobial resistance

#### ANTI-MICROBIAL PROPERTIES ON DIFFERENT SOLVENT EXTRACTS OF FOUR HYOSCYAMUS SPECIES FROM TURKEY

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#### Background

*Hyoscyamus* species contain important tropane alkaloids, such as Hyoscyamine, scopolamine and to lesser extend atropine which have antispasmodic, anticholinergic, analgesic and sedative effects. These compounds are produced mainly by *Hyoscyamus albus* L. This shrub is able to accumulated high amount of tropane alkaloids and these alkaloids indicated antimicrobial activity to microorganisms.

#### Objectives

In this study obtained extracts of some plant species which belongs to *Hyoscyamus* (*Hyoscyamus reticulatus* L., *Hyoscyamus leptocalyx* STAPF, *Hyoscyamus aureus* L., *Hyoscyamus albus* L.) (Solanaceae) family by various solvent systems were researched within the context of their antimicrobial activities.

#### Methods

Antimicrobial activities of hexane, ethylacetate and methanol extracts of *Hyoscyamus* species were tested by disc diffusion method. Gram positive and gram negative standard bacteria strains, clinical isolates and *Candida albicans* (ATCC-10231) yeasts over amoxicillin/clavulanic acid (2:1) ofloxacin, nystatin, imipenem and netilmicin standart antibiotics and 150 µg/6 mm paper disc plant extracts tested.

#### Conclusions

The antimicrobial activity of hexane, ethylacetate and methanol extracts of *Hyoscyamus* species showed different antibacterial activity against tested microorganisms. The diameters of growth inhibition zones of *Hyoscyamus* species ranged from 8 mm to 12 mm. Among all the *Hyoscyamus* extracts studied, methanol extract of *H. reticulatus* and *H. aureus* demonstrated the highest antimicrobial activity versus *S. aureus* (clinic-isolate). *S. aureus* (ATCC-25923), *S. aureus* (clinic-isolate),

and *S. pyogenes* (ATTC-19615) were sensitive to all *Hyoscyamus* extracts except *H. leptocalyx*.

**FEMS-2344**

**Antimicrobial resistance**

**ANTIMICROBIAL RESISTANCE- AND CONSUMPTION SURVEILLANCE – A 12-YEAR HOSPITAL EXPERIENCE**

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**Background**

During the last years surveillance of Antibiotic resistance (AR) and Antibiotic consumption (AC) became a part of the efforts to contain AR.

**Objectives**

Our aim was to analyze the results of 2002 – 2014 AR and AC surveillance, conducted at a multiprofile tertiary care 300-bed hospital in Sofia.

**Methods**

Etiology of infections and AR were monitored as a part of the national surveillance program BulSTAR. AC was calculated by ABC calc (D. Monnet).

Results. A total of 44786 clinically significant bacteria have been isolated. The first 3 top isolates from urine were *Escherichia coli* (Eco), other *Enterobacteriaceae* (oE-ae), *Enterococcus* spp (Enc); from wounds - *Staphylococcus aureus* (Stau), Eco, Enc; from bacteremia - Eco and oE-ae, Stau and Enc; from the respiratory tract – *Streptococcus pneumoniae* (Spn) and *Haemophilus influenzae* (Hin). The rate of MRSA from wounds was 11 %, but from bacteremia 50 % to 78 %. The most problematic organisms were E-ae with ESBL: they increased from 3.8 % in 2002 through 12 % in 2007 till 26.05 % in 2014. Among Enc there were not VRE; among Spn – the rate of PNSSP was 16.4 %; 10 % of Hin were bla (+). The results from AC showed a fluctuation from 27 to 54 DDD/100 bed-day, with a significant higher AC in the surgical ICU (~ 250 DDD/100 bed-day).

**Conclusions**

Our results illustrated an increase of antimicrobial resistance (ESBL producing *Enterobacteriaceae*) despite the antibiotic policy attempts and suggest for more active actions both nationally and globally.

**FEMS-1496**

**Antimicrobial resistance**

## **AMINO ACID SUBSTITUTIONS IN GYRA AFFECTS QUINOLONE SUSCEPTIBILITY IN *SALMONELLA* TYPHIMURIUM**

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### **Background**

Amino acid substitutions have generally been found within the quinolone resistance determining regions in subunit A of DNA gyrase (GyrA) of *Salmonella* Typhimurium, but direct evidence of the contribution of these substitutions to quinolone resistance remains to be shown.

### **Objectives**

To clarify the significance of amino acid substitutions in *S. Typhimurium* GyrA to quinolone resistance, we expressed recombinant wild-type (WT) and S83F, D87N, D87G, D87Y and S83F-D87N mutant gyrases in *Escherichia coli* and characterised them *in vitro*.

### **Methods**

WT and mutant gyrases were reconstituted *in vitro* by mixing recombinant subunits A and B. Correlation between the amino acid substitutions and resistance to quinolones ciprofloxacin, levofloxacin, nalidixic acid and sitafloxacin was assessed by the quinolone-inhibited supercoiling assay.

### **Conclusions**

We concluded that amino acid substitutions at position 83 and 87 of GyrA, and quinolone structural variations at position 8 influenced the interaction between DNA gyrases and drugs. Furthermore, we suggest that sitafloxacin might be a choice for the treatment of salmonellosis caused by ciprofloxacin-resistant *S. Typhimurium*.

**FEMS-0857**

**Antimicrobial resistance**

## **PHYSIOLOGICAL CHARACTERIZATION OF SHV14 BETA-LACTAMASE OF KLEBSIELLA PNEUMONIAE**

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### **Background**

*Klebsiella pneumoniae* (KP) is an opportunistic pathogen of *Enterobacteriaceae* family and is resistant to many antibiotics, especially the beta-lactams, due to extensive production of beta-lactamases. SHV-14 is one of the potent extended-spectrum serine beta-lactamases present chromosomally in *K. pneumoniae*.

### **Objectives**

Physiological characterization of the membrane bound form of *bla*SHV-14 in *K. pneumoniae* and study its effect on antibiotic resistance and biofilm formation.

### **Methods**

Membrane bound form of *bla*SHV14 gene was cloned in pBAD-18 Cam vector and transformed in *ampC* deleted CS109 *Escherichia coli* strain for *in vivo* studies. Minimum Inhibitory Concentration (MIC) was determined in accordance with Clinical and Laboratory Standards Institute (CLSI) using the micro-broth dilution technique. Beta-lactamase activity of cloned *bla*SHV14 was checked by monitoring hydrolysis of a chromogenic substrate nitrocefin at particular time interval in *E.coli* CS109 cell lysate. Biofilm formation on polystyrene surface was also studied by staining the biofilms formed with 0.1% crystal violet stain. An elevated level of beta-lactamase hydrolytic activity was observed against penicillin and cephalosporin group of antibiotics in the presence of *bla*SHV14 in *ampC* deleted CS109. Beta-lactam resistance was increased by eight fold against cephalosporin and 14 fold against penicillin group of antibiotics as compared to the control lacking *bla*SHV14 gene. Biofilm formation index of *ampC* deleted CS109 harbouring *bla*SHV14 gene showed three fold increments as compared to control devoid of *bla*SHV14 beta-lactamase.

### **Conclusions**

Therefore, the presence of *bla*SHV14 gene in *Klebsiella pneumoniae* makes the cell resistant to beta-lactam group of antibiotics and subsequently enhances biofilm formation.

**FEMS-2938**

**Antimicrobial resistance**

## **ESCHERICHIA COLI FROM BLUE MUSSELS (MYTILUS EDULIS) AS INDICATORS FOR ANTIBIOTIC RESISTANCE**

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### **Background**

Antibiotic resistance in bacteria is an increasing challenge in all situations where such agents are applied. Blue mussels (*Mytilus edulis*) are suspension feeders and may retain particles with close to 100% efficiency. An adult blue mussel (60 mm in shell length) may clear between 12 and 240 litre of seawater per day for particles containing bacteria. According to the current EU regulations (EU Directive 854/2004), farm sites for cultivation of bivalves must be classified according to their suitability in terms of microbiological and chemical water quality. One of the parameters to be examined is the concentration of *Escherichia coli* in the soft parts and mantle water of the bivalves.

### **Objectives**

*E. coli* derived from animals or man by sewage or land runoff, may subsequently be filtered out by Blue mussels and can be used as indicators for the antibiotic resistance status and detect spatial and temporal changes in resistance prevalence.

### **Methods**

The Norwegian Food Safety Authority submit annually approximately 300 samples of Blue mussels from over 35 stations along the coast. The number of *E. coli* in these samples are determined by a multiple tube dilution method (Donovan's method, ISO 16649-3). On average 60% of the examined samples harbour *E. coli*, and strains are currently being isolated, and will be characterised.

### **Conclusions**

Isolation and characterisation of the antibiotic resistance profile of *E. coli* from Blue mussels may represent a potential indicator of the long term changes.



**FEMS-2229**

**Antimicrobial resistance**

## **CARRIAGE OF CARBAPENEM-RESISTANT ENTEROBACTERIACAE IN HOSPITAL IN-PATIENTS WITH DIARRHOEA**

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### **Background**

Due to the rising incidence of carbapenem-resistant Enterobacteriaceae (CRE) in the UK and the increased mortality associated with such infections, it is imperative that adequate infection control measures are in place to limit their spread in hospitals. Despite targeted screening of patients at increased risk of carriage, the overall burden may be underestimated. Thus it is recommended that healthcare facilities with CRE conduct surveillance studies to establish the true burden of infection.

### **Objectives**

Surveillance was performed using diarrhoeal stools from in-patients at a large London teaching hospital to assess whether current screening strategies adequately identify carriers, particularly those posing an increased risk of dissemination.

### **Methods**

In addition to routine investigations, diarrhoea stool samples from in-patients were cultured onto Brilliance CRE medium. Blue or pink pigmented colonies that developed overnight were sub-cultured onto MacConkey agar with an ertapenem disc. Ertapenem-resistant colonies were identified by VITEK 2. *Enterobacteriaceae* with minimum inhibitory concentration values above the screening threshold for meropenem were referred to the national reference laboratory for further testing.

### **Conclusions**

Over seven months, 1013 stool samples from 899 patients were screened. Thirteen colonised patients were identified; a carriage rate of 1.45%. Two carbapenemase producing *Enterobacteriaceae* (CPE) and 11 extended spectrum or AmpC  $\beta$ -lactamase producers with porin loss were isolated. Screening of diarrhoea stools detected a higher proportion of carbapenemase non-producing CRE than routine surveillance swabs (11/889 vs. 4/8663;  $P=0.001$ ). CPEs remain rare in acute healthcare settings, even among patient populations with increased risk of transmission. Current guidance for CRE surveillance is considered adequate.





**FEMS-0858**

**Antimicrobial resistance**

## **BETA-LACTAMASES INFLUENCE ESCHERICHIA COLI BIOFILM FORMATION**

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### **Background**

Drug resistance mechanisms of bacteria are a menace to antibiotic therapy. Antibiotic inactivating enzymes and biofilm formation are prominent bacterial escape routes. The largest group of drug hydrolysing enzymes are beta-lactamases which may influence biofilm formation.

### **Objectives**

Inducible beta-lactamase expression is regulated by the same genes that govern peptidoglycan recycling. We attempt to elucidate the role of these genes in biofilm formation by the beta-lactamase producing *E. coli*.

### **Methods**

Single and double gene deletion mutants of *ampC*, *ampD*, *ampE* and *ampG* in *E. coli* 25113 were created by the one-step gene inactivation method. Serine beta-lactamases (TEM-1, OXA-2, CTX-M-15 and SHV-14) from the nosocomial enterobacterial isolates were cloned and expressed in the parent and mutant strains. The influence of deletions on beta-lactamase expression and subsequent effect on biofilm formation were studied by chromogenic cephalosporin hydrolysis and crystal violet staining, respectively. The drug resistance alteration was enumerated by standard microbroth dilution techniques. It was observed that serine beta-lactamases like TEM-1 and OXA-2 lowered the biofilm formation whereas ESBLs like CTX-M-15 and SHV-14 facilitated it. Deletion of *ampD* showed varied levels of beta-lactamase expression and similar effect was observed in biofilm formation. Nonetheless, overexpression of beta-lactamases in  $\Delta$ *ampE* enhanced biofilm formation by 45% whereas a biofilm reduction of 29% was observed in  $\Delta$ *ampG* mutant as compared to beta-lactamase deficient strain.

### **Conclusions**

We infer that beta-lactamase expression not only enhances beta-lactam resistance but also influences biofilm formation; and the genes *ampD*, *ampE* and *ampG* affect the expression of Class A and D beta-lactamases in *E. coli*.



**FEMS-0899**

**Antimicrobial resistance**

**FIRST REPORT OF THE PRESENCE OF OXACILLINASES AND METALLO-BETA-LACTAMASES IN INVASIVE ISOLATES OF CARBAPENEM-RESISTANT ACINETOBACTER BAUMANNII IN VOJVODINA PROVINCE, SERBIA**

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**Background**

*Acinetobacter spp.* are the most common invasive isolates from blood in Vojvodina Province. The importance of these microorganisms is an exceptional ability of survival and spread in the hospital environment, as well as the extraordinary capacity to quickly acquire different mechanisms of resistance to a wide variety of antimicrobial agents, including carbapenems.

**Objectives**

To detect the presence of encoded genes for oxacillinases and metallo- $\beta$ -lactamases in carbapenem resistant isolates of *Acinetobacter baumannii* in Vojvodina Province.

**Methods**

The identification to the species level of *Acinetobacter baumannii* was confirmed by PCR detection of *bla*<sub>OXA-51-like</sub>. In 50 carbapenem-resistant *Acinetobacter baumannii* isolates, detection of *bla*<sub>OXA</sub> and *bla*<sub>MBL</sub> gene was conducted by multiplex PCR, as well as the detection of insertion sequences IS*Aba1*.

**Conclusions**

*bla*<sub>OXA-24-like</sub> was detected in 45 carbapenem-resistant *Acinetobacter baumannii* isolates. Twelve isolates were positive for *bla*<sub>OXA-58-like</sub>. The presence of *bla*<sub>OXA-23-like</sub> gene was not detected in any of the isolates. A simultaneous presence of two genes was detected in 43 isolates. Thirty eight isolates were positive for *bla*<sub>OXA-24-like</sub> and *bla*<sub>OXA-51-like</sub> genes and in 5 isolates *bla*<sub>OXA-58-like</sub> and *bla*<sub>OXA-51-like</sub> genes were detected. Seven isolates had three genes, *bla*<sub>OXA-24-like</sub>, *bla*<sub>OXA-51-like</sub> and *bla*<sub>OXA-58-like</sub>. All isolates were PCR positive for IS*Aba1*. The presence of *bla*<sub>IMP</sub> and *bla*<sub>VIM</sub> was not detected in any of the isolates. The resistance to carbapenems in isolates of *Acinetobacter baumannii* in Vojvodina Province is caused by production of OXA-24 and OXA-58 oxacillinases, because metallo- $\beta$ -lactamases were not detected.

## **FEMS-2098**

### **Antimicrobial resistance**

#### **COMPARATIVE EFFICACY OF THREE CHLORINE-BASED DISINFECTANTS TO CONTROL PLANKTONIC AND SESSILE ESCHERICHIA COLI**

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### **Background**

Sodium hypochlorite (SH) is the most common antimicrobial agent used in food industry even if it produces unhealthy and toxic by-products. New chlorine-based decontamination strategies are emerging for the decontamination of fresh products and disinfection of surfaces, such as neutral electrolyzed oxidizing water (NEOW) and chlorine dioxide (CD).

### **Objectives**

The aim of the present work was to assess the effects of three chlorine-based disinfectants (SH, NEOW and CD) on the control of *Escherichia coli* in both planktonic and sessile states. The stability of the disinfectants was also assessed over time.

### **Methods**

Four different concentrations (20, 50, 80 and 100 ppm) were tested on the control of planktonic and sessile *E. coli* cells. Planktonic tests were made by measuring growth inhibition over time. Biofilms were formed on stainless steel surfaces and the efficacy of chlorine-based products was assessed by colony forming units counting. The free chlorine content was also measured over time for each disinfectant tested.

### **Conclusions**

NEOW was the most efficient disinfectant in the control of both planktonic cells and biofilms while SH was the least effective. In terms of free chlorine content in solution/disinfectant stability, SH was the most instable and NEOW was the most stable. In conclusion, NEOW is an effective disinfectant strategy for the control of both planktonic and sessile cells and disinfectant decay was negligible compared to the other disinfectants.

## **FEMS-1014**

### **Antimicrobial resistance**

#### **IDENTIFICATION AND EVALUATION OF PEPTIDES FOR ACTIVE AND PASSIVE IMMUNIZATION AGAINST PSEUDOMONAS AERUGINOSA**

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#### **Background**

*Pseudomonas aeruginosa* is a Gram-negative bacterium, commonly present in the environment, that causes serious health problems when it infects humans and is resistant to many antibiotics and treatments. Patient groups at risk for acquisition of *P. aeruginosa* infections include those with cystic fibrosis (which almost always leads to chronic airway infection), paraplegic, burn and immune systems deficiencies patients. Prevention of infection by vaccination is desirable until today no vaccine has yet obtained market authorization.

#### **Objectives**

This project is aimed at the development of the first peptide-based *P. aeruginosa* vaccine and to test planned techniques. Our goal is to produce high immunogenic response in human body.

#### **Methods**

We have selected short peptides from *P. aeruginosa* surface localized proteins using bioinformatic tools. Now we are using genetic engineering and cloning to put those peptides into stable protein scaffolds. After stability tests we will try to induce response in animal model and obtain antibodies for further testing with serum samples from cystic fibrosis patients and *Pseudomonas aeruginosa* clinical isolates. To evaluate whether the specific antibodies are protective *in vitro*, we will use opsonophagocytosis assays.

Finally, we will perform *in vivo* infection experiments in animal models after immunization with targets to reveal whether the produces targets are protective against pathogen.

#### **Conclusions**

As this is rather new development method, we are facing problems and we are trying to establish protocols for our purpose, nevertheless project is going forward and we are expecting successful results soon.

**FEMS-0772**

**Antimicrobial resistance**

**ANTIMICROBIAL ACTIVITY OF NEWLY SYNTHESIZED NI(II) COMPLEXES ON LABORATORY STRAINS OF MICROORGANISMS AND CLINICAL ISOLATES**

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**Background**

The escalation of multidrug-resistant (MDR) bacteria is documented to be a serious problem that affects the choice of appropriate antibiotic therapy and increases the probability of unfavorable infection outcome. Resistance has emerged towards all classes of antibiotics leading to a continuous need for producing new drugs. One of the promising methods in coping with bacterial resistance is the use of alternative classes of antimicrobial agents.

**Objectives**

The aim of this study was to investigate the antimicrobial activity of seven newly synthesized Ni(II) complexes.

**Methods**

Minimum inhibitory concentrations (MICs) of complexes were determined by broth microdilution method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2007). The antimicrobial activity was investigated on eight laboratory control strains of microorganisms: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 1228, *Bacillus subtilis* ATCC6633, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella abony* NCTC 6017, yeast *Candida albicans* ATCC 10231, and twenty-five clinical isolates of *Escherichia coli* and *Pseudomonas aeruginosa*. All tests were performed in Müller Hinton broth for the bacterial strains and in Sabouraud dextrose broth for the yeast. Complexes were dissolved in 1% dimethylsulfoxide (DMSO) and then diluted to the desired concentrations ranging from 31–1000 micrograms/ml with addition of 0.05% triphenyl tetrazolium chloride as a growth indicator.

**Conclusions**

Two of tested compounds exhibited strong activity against Gram-positive bacteria, moderate activity against standard strains of Gram-negative bacteria and weak activity against clinical isolates of *Escherichia coli*.

**FEMS-3097**

**Antimicrobial resistance**

## **ELUCIDATING THE ROLE OF UPC2 AND NDT80 TRANSCRIPTION FACTORS IN CANDIDA PARAPSILOSIS AZOLE RESISTANCE**

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### **Background**

*C. parapsilosis* is responsible for hospital-acquired infections with remarkable risks especially to neonates and immunocompromised patients. In the past decades, this human pathogen has been ranked as second or third most isolated fungus, depending on the geographical region. Since its incidence is growing, the number of *C. parapsilosis* resistant to azoles has been followed the same trend. Despite *C. parapsilosis* high prevalence, molecular mechanisms associated to azole resistance remain poorly studied. In a pioneer study addressing azole resistance, an overexpression of ergosterol biosynthesis genes, namely *ERG11*, and their regulators, *UPC2* and *NDT80* genes, were identified as main players in posaconazole resistance.

### **Objectives**

The aim of this work was to reveal the importance of *UPC2* and *NDT80* transcription factors in *C. parapsilosis* posaconazole resistance, through control of ergosterol biosynthesis.

### **Methods**

For that, both genes were independently and simultaneously knockout in posaconazole resistant strain BC014R<sub>PSC</sub>, using *SAT1*-Flipper cassette. Thus, *upc2Δ*, *ndt80Δ* and *upc2Δ/ndt80Δ* mutants were generated. These strains were characterized regarding their azole susceptibility profile.

### **Conclusions**

Deleted strains became susceptible to posaconazole, voriconazole and fluconazole. Minimal Inhibitory Concentrations for azoles in *upc2Δ* and *upc2Δ/ndt80Δ* mutants were similar and inferior to those obtained in *ndt80Δ* strain. We conclude that *UPC2*



and *NDT80* genes are both involved in *C. parapsilosis* azole resistance, however the first seems to play a major role.

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**FEMS-2466**

**Antimicrobial resistance**

**PATTERN OF ANTIMICROBIAL RESISTANCE OF BRUCELLA SPECIES  
ISOLATED FROM CLINICAL SPECIMENS OF BRUCELLOSIS  
PATIENTS, KASHAN-IRAN**

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**Background**

Brucellosis is an important health problem in our country in aspect of long lasting treatment and tendency to relapse. Getting information about the pattern of brucella antibiotic susceptibility help us to treat patients more successfully

**Objectives**

This study was done to assess antibiotic susceptibility pattern of brucella with E-test method in Kashan region

**Methods**

This descriptive study was performed on 48 clinical samples were positive culture for brucella in Kashan shahid Beheshti hospital in 2013. Clinical samples got from patients suspicious to brucellosis and Minimum inhibitory concentrations was calculated for ciprofloxacin, doxycycline, rifamin , azithromycin, ceftriaxone, streptomycin and cotrimoxazol with E-test. For each sample a form contain of information about age, sex, clinical manifestation and E-test results was filled. All informations were entered in SPSS 16 software. Results were shown as descriptive statistics.

**Conclusions**

All brucella isolates were sensitive to antibiotics, without any report of resistance. Cotrimoxazol with MIC<sub>50</sub>:0.016, MIC<sub>90</sub>:0.064 was the most effective choice. Doxycycline the second one, streptomycin had the highest MIC. There was not any statistical relation between patient age, sex, type of reception and antibiotic susceptibility.

Cotrimoxazol and doxycycline as effective drugs on brucella are the best choice for treatment of brucellosis in our region. Streptomycin should be used cautiously because it had the highest MIC against brucella spp in our study.

## **FEMS-2551**

### **Antimicrobial resistance**

#### **PHENOTYPIC RESISTANCE TO ANTIBIOTICS IN STAPHYLOCOCCUS AUREUS STRAINS ISOLATED FROM PUBLIC HEALTH STUDENTS.**

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#### **Background**

*Staphylococcus aureus* is an opportunistic pathogen that colonizes the anterior nares and skin, being one of the most common causes of skin, soft tissue and nosocomial infections.

#### **Objectives**

This study was undertaken to evaluate *S. aureus* carriage in public health students at the Faculty of Pharmacy (Granada, Spain), and phenotypical resistance of the strains to antibiotics.

#### **Methods**

This study was performed on 144 public health students. Sampling was done once between October 2013 and January 2014. Samples were collected from both nares inserting a sterile nasals wab into nostril. The swabs were cultured on Mannitol Salt Agar and incubated at 37°C for 24h. Bacterial identification followed the standard phenotypic methods. Antibiotic susceptibility of isolates was tested by disc diffusion method (EUCAST, 2013). A wide array of antibiotics representing the main groups: penicillins, cephalosporins, aminoglycosides and others.

#### **Conclusions**

*S. aureus* was isolated from 20% (29/144) of the student population. Regarding the penicillin group, 3 of 29 strains did not show resistance to this group. 82.7% of *S. aureus* positive strains demonstrated multiresistances to penicillins and cephalosporins. Among the group of aminoglycosides, 93.1% of strains were multiresistant to them, and 2 strains were found to be sensitive for all antibiotics in this group. Furthermore, all strains of this study proved resistances to nalidixic acid. We may conclude that the strains studied exhibit a broad spectrum of multiresistances, which suggests that the use of some penicillins, cephalosporins and aminoglycosides antibiotics could be ineffective for the treatment of infections caused by *S. aureus*.



## **FEMS-1896**

### **Antimicrobial resistance**

#### **ANTIMICROBIAL RESISTANCE AND MOLECULAR CHARACTERIZATION OF AVIAN PATHOGENIC ESCHERICHIA COLI IN BELGIUM.**

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#### **Background**

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis and significant economic losses in the poultry industry.

#### **Objectives**

To isolate, perform antimicrobial susceptibility testing, serotyping, phylogrouping and molecular characterization of the APEC isolates from Belgium.

#### **Methods**

Antimicrobial susceptibility was tested by micro-broth dilution (TREK diagnostics sensititre plates) using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Multi drug resistance (MDR) was described as resistance to more than three classes of antimicrobials. O-antigen serotyping was done using 31 different antisera (LREC, Spain). Strains negative for all these serotypes were considered not typeable (NT). The isolates were grouped into A, B1, B2 and D phylo-groups. The microarray (CLONDIAG GmbH, Jena, Germany) system was used to test for virulence and antimicrobial resistance genes.

#### **Conclusions**

The 212 APEC isolates were resistant to ampicillin (61%), ciprofloxacin (58%) and tetracycline (49%). MDR was observed in 135 (64%) of the isolates. 13 (6%) isolates have been presumptively identified as ESBL producing. O-antigen grouping showed that 73 (34%) isolates collectively belonged to sero-group O78, O2, and O1; 65 (31%) to other sero-groups associated with colibacillosis in poultry, and 74 (35%) were NT. The most prevalent phylo-groups were D (42%) and A (40%); 15% were phylo-group B1, while 3% were B2. Genes coding for adhesion factors *fimH* (75%) and Iron acquisition systems *iroC* (57%), *irp2* (52%), were mostly observed.

Antimicrobial resistance is high and a large percentage of the isolates are MDR. APEC strains showed high prevalence of virulence factors that might be associated with greater pathogenic potential.

## **FEMS-0992**

### **Antimicrobial resistance**

#### **CLASS-I AND CLASS-II INTEGRONS IN MULTIDRUG RESISTANT ESBL AND CARBAPENAMASE PRODUCING ENTEROBACTERIACEAE FROM EGYPT**

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### **Background**

*Escherichia coli* and *Klebsiella pneumoniae* with Extended-Spectrum- $\beta$ -Lactamase- (ESBL) and carbapenemase-mediated resistance are increasingly reported in hospital and community settings worldwide. ESBL CTX-M-15 and Class-D carbapenemase OXA-48 co-production, plus resistance to other antibiotics, limit treatment options, while integrons support resistance genes expression and movement.

### **Objectives**

To characterize genetic determinants encoding  $\beta$ -lactam resistance in Enterobacteriaceae isolates collected from Egypt, and investigate their association with class-I and class-II integrons.

### **Methods**

One hundred and sixty-nine ESBL-producing *E. coli* and *K. pneumoniae* from nosocomial and community-acquired infections were collected from 10 Egyptian university hospitals in two surveillance studies (Group-1: 65 *K. pneumoniae* 2000-2003, Group-2: 41 *E. coli*, 63 *K. pneumoniae* 2009-2011). Resistance genes and integron integrases were identified by PCR and DNA sequencing. Plasmid located blaCTX-M-15 was identified in 11% (n=7) of isolates from group-1 vs. 97% (n=101) from group-2. Non-metallo-carbapenemase blaOXA-48 was detected in 88% (n=57) of isolates from group-1 vs. 95% (n=99) from group-2. Plasmids displayed a range of different RFLP patterns. Integrase-1 was detected in 83% (n=54) of isolates from group-1 vs. 93% (n=97) from group-2, while Integrase-2 was identified in 97% (n=63) of isolates from group-1 isolates vs. 89% (n=93) from group-2.

### **Conclusions**

We have shown that association between integrases and resistance genes carriage appear to increase over time. The linkage between integrases and blaCTX-M-15, and blaOXA-48 is currently being determined by investigating the first gene cassettes in integrons.



## FEMS-2750

### Antimicrobial resistance

#### FIRST DESCRIPTION OF A FOSFOMYCIN RESISTANT CLINICAL ESCHERICHIA COLI ISOLATE HARBOURING PLASMID-BORNE FOSA3 AND BLACTX-M-15 IN EUROPE

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#### Background

The widespread of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* has reignited the interest in old antibiotics, being fosfomycin one of the first therapeutic options for treatment of community acquired urinary tract infections. Resistance to fosfomycin is mostly linked to chromosomal mutations causing reduced fosfomycin uptake, though acquisition of plasmid-mediated fosfomycin resistance genes (*fos*), mainly *fosA3* in isolates from Asia and non-clinical settings has been reported.

#### Objectives

To assess the frequency of plasmid-mediated fosfomycin resistance genes and their genetic background among fosfomycin resistant *Enterobacteriaceae* isolates from a community laboratory from Portugal.

#### Methods

Between August 2012-September 2013, 58 fosfomycin resistant isolates (29 *K.pneumoniae*, 26 *E.coli*, 2 *Proteus mirabilis* and 1 *Morganella morganii*) were identified among 461 ESBL-producing *Enterobacteriaceae*. Antibiotic susceptibility testing was performed by standard methods, including fosfomycin disks supplemented with glucose-6-phosphate. *fos* (*fosA*, *fosA3*, *fosB*, *fosC2*), *bla*<sub>ESBL</sub> and *rmtB* genes were screened by PCR and sequencing. *E. coli* phylogroups and genetic context were investigated by PCR and PCR mapping and sequencing, respectively. Plasmid typing was assessed by PBRT, IncFII typing (FAB formula), I-CeuI-PFGE and hybridization.

#### Conclusions

Although with a low prevalence, we identified in the urine of a 61 year old patient, one *E. coli* (1.7%) isolate of phylogenetic group D<sub>1</sub>, from carrying the *fosA3* gene together with *bla*<sub>CTX-M-15</sub> in an F2:A6:B- IncFII plasmid, which corresponds to the first description of *fosA3* in Europe. Although *aac-lb-cr*, *rmtB* and *bla*<sub>OXA-1</sub> genes were not detected, the *fosA3* gene was located in an IS26-composite transposon, similar to others previously reported in Asia suggesting a common platform.

**FEMS-3114**

**Antimicrobial resistance**

**IN VITRO ACTIVITY OF AQUEOUS AND METHANOLIC EXTRACTS OF PSIDIUM GUAJAVA, NAUCLEA LATIFOLIA AND BROPHYLLUM PINNATUM AGAINST BACTERIA ISOLATED FROM CHICKEN MEAT**

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**Background**

medical knowledge derived from traditional societies has motivated searches for new bioactive molecules derived from plants that show potent activity against bacterial pathogens.

**Objectives**

The present study investigated the antibacterial activity of *Psidium guajava*, *Nauclea latifolia* and *Brophyllum pinnatum* on *Staphylococcus aureus*, *Escherichia coli* and *Salmonella* isolated from fresh and frozen chicken meat sold in Ibadan metropolis.

**Methods**

The samples were processed for isolation, identification and antibiotics susceptibility. The phytochemical components of aqueous and methanolic extracts of the three plants were obtained using column chromatography. Antimicrobial activity of the plants extracts was done using agar well diffusion method.

**Conclusions**

Most of the bacterial isolates were resistant to four or more commonly used antibiotics. All the plant extracts exhibit antibacterial activity; there was a significant difference in the antibacterial activity of the methanolic extract of the plants studied ( $P < 0.05$ ). Among the bacteria tested *Escherichia coli* was the most sensitive while *Psidium guajava* showed extensive inhibition zone. This study reveals that there is high prevalence of multi-drug resistant bacteria in the chicken meat tested and that *Psidium guajava* is potential source of antibiotics for drug development against the bacteria studied.

**FEMS-2974**

**Antimicrobial resistance**

## **IN VITRO SYNERGISTIC EFFECTS OF COLISTIN, TIGECYCLINE, TOBRAMYCIN AND DORIPENEM ALONE OR IN COMBINATIONS AGAINST CARBAPENEM-RESISTANT ENTEROBACTERIACEAE STRAINS**

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### **Background**

The emergence of carbapenem resistance in *Enterobacteriaceae* is an important threat to global health. The reason of prevalence of bacterial resistance to antibiotics continues to increase, infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE) are responsible for high rates of mortality and represent a major therapeutic challenge.

### **Objectives**

The aim of this study was to investigate effective antibiotic combinations against carbapenem-resistant *Enterobacteriaceae* (CRE) strains.

### **Methods**

The minimum inhibitory concentrations (MIC) of doripenem, colistin, tigecycline and tobramycin were determined by microbroth dilution method against 39 clinical strains isolated from patients in the intensive care unit (ICU). Carbapenem resistance mechanisms in these isolates were investigated by PCR technique. The in vitro synergistic activities of tigecycline, colistin, tobramycin and doripenem in double combination were determined by microbroth chequerboard technique and results were interpreted using the fractional inhibitory concentration (FIC) index.

### **Conclusions**

Based on MIC<sub>90</sub>s, colistin (1 mg/l) was the most potent agent, followed by tigecycline (2 mg/l), tobramycin (32 mg/l), and meropenem (32 mg/l). According to PCR studies, carbapenem resistance in tested *Enterobacteriaceae* isolates is most often mediated by OXA-48 type carbapenemases. Additionally six isolates were positive for the

genes encoding VIM and one isolates was positive for the genes encoding KPC. Given that a FIC index of  $\leq 0.5$  is borderline, synergistic interactions were most frequent with tigecycline-tobramycin (31 %). No antagonism was observed. The findings of this study may play a useful role in selecting the appropriate combinations when a single agent is inadequate.

**FEMS-1536**

**Antimicrobial resistance**

## **SYNERGISTIC EFFECT OF NATURAL COMPOUNDS ON ANTIBIOTICS AGAINST ACINETOBACTER BAUMANNII**

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### **Background**

Difficulties involved in treating drug-resistant pathogens have created a need for new therapies.

### **Objectives**

In this study, we investigated the possibility of natural compounds as an adjuvant for antibiotics against *Acinetobacter baumannii*.

### **Methods**

Measurement of the fractional inhibitory concentration (FIC) for OA demonstrated that it only synergizes with aminoglycoside antibiotics. Other classes of antibiotics (e.g., ampicillin, rifampicin, norfloxacin, chloramphenicol, and tetracycline) have additive effects with OA. Unlike OA, HBA and PP synergize only with chloramphenicol antibiotics. Microarray and quantitative reverse transcription-PCR analysis under OA indicated that genes involved in ATP synthesis and cell membrane permeability, the gene encoding glycosyltransferase, peptidoglycan-related genes, phage-related genes, and DNA repair genes were upregulated. OA highly induces the expression of *adk*, which encodes an adenylate kinase, and *des6*, which encodes a linoleoyl-CoA desaturase, and deletion of these genes increased FICs; these observations indicate that *adk* and *des6* are involved in the synergism of OA with aminoglycosides. Data obtained using 8-anilino-1-naphthalenesulfonic acid, fluorescence-conjugated gentamicin, and membrane fatty acid analysis indicates that *adk* and *des6* are involved in changes in membrane hydrophobicity and permeability. Proton-motive force and ATP synthesis tests show that those genes are also involved in energy metabolism.

### **Conclusions**

We screened 28 natural compounds from plants and confirmed that a pentacyclic triterpenoid, oleanolic acid (OA) could be used only with aminoglycoside antibiotics and other natural compounds (4-hydroxybenzaldehyde and propyl 4-hydroxybenzoate) could be used only with chloramphenicol antibiotics. Taken together, our data show that OA boosts aminoglycoside uptake by changing membrane permeability and energy metabolism in *A. baumannii*.

## **FEMS-2558**

### **Antimicrobial resistance**

#### **ANTIBIOTIC SUSCEPTIBILITY TESTING OF *SALMONELLA* ENTERITIDIS ISOLATES FROM RAŠKA REGION OF SERBIA**

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#### **Background**

*Salmonella* spp. are zoonotic agents widely spread amongst poultry, cattle and swine which are marked as one of the leading causes of gastroenteritis in humans in the modern world. Today's open market and trade between countries are main reasons of propagation of *Salmonella* serotypes worldwide.

Heavy use of antibiotics in raising animals leads to selective pressure to *Salmonella* serotypes, allowing propagation of resistant serotypes and development or acquisition of genes, gene complexes or mutations which are holders of resistance. Multiresistant *Salmonella* serotypes can transfer resistance genes to other bacterial species.

#### **Objectives**

The aim of this investigation was to determine dominant *Salmonella* serotypes in poultry production in the region of Raška in Serbia and check their resistance to the commonly used antibiotics.

#### **Methods**

*Salmonella Enteritidis* was determined as one of dominant serotypes in the investigated period (2010/2011). Resistance testing was conducted on 41 *Salmonella Enteritidis* isolates. Test method was NLCCS Disk Diffusion Method. Antimicrobial panel comprised of 16 antibiotics.

#### **Conclusions**

Most of the samples were sensitive to all 16 antibiotics. Five isolates were resistant to flumequine and nalidixic acid. Six isolates showed intermediate sensitivity to neomycin and two were intermediate sensitive to tetracycline. This result is not surprising considering fact that these antibiotics are commonly used in poultry production in Serbia. These results suggest that it would be necessary to reassess use of flumequine and limit its use only to cases in which the causative agent had

exhibited resistance to other antibiotics and the bacteriological confirmation of diagnosis and susceptibility testing of pathogens justifies its application.

**FEMS-1903**

**Antimicrobial resistance**

**A NEW PROMOTER FOR EXPRESSION OF THE AAC(6')-IB-CR GENE IN CLASS 1 INTEGRONS.**

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**Background**

Class 1 integrons are genetic elements able to acquire, exchange and express gene cassettes mainly encoding antibiotic resistance. GCs are usually promoterless and are expressed from a common promoter Pc. Thirteen Pc variants of different strenghts have been described, including a weak (PcW) variant. The *aac(6')-Ib-cr* gene, conferring resistance to aminoglycosides, norfloxacin and ciprofloxacin, is mostly found at first position in class 1 integrons. A 101-bp sequence upstream of *aac(6')-Ib-cr*, contains a putative promoter (named P<sub>A</sub> in this study) suggesting a an expression control by both Pc and P<sub>A</sub>.

**Objectives**

The role of respectively Pc and P<sub>A</sub> in expression of *aac(6')-Ib-cr* will be evaluate.

**Methods**

A 484-bp fragment, from a clinical class 1 integron (*Klebsiella pneumoniae* C1911) and containing the PcW variant, the P<sub>A</sub> promoter and the first 194-bp of the *aac(6')-Ib-cr* gene cassette, was cloned upstream the *lacZ* reporter gene. Wild-type and mutated promoters-were used. Beta-galactosidase assays and quantification of *lacZ* transcripts were used to estimate the level of *aac(6')-Ib-cr* expression. Mapping of the transcription start site by 5' RACE and characterization the N-terminal amino acids of the protein are in progress.

**Conclusions**

**Both promoters are involved in the transcription of *aac(6')-Ib-cr*. However, the level of transcription is slightly increased (12% with begal assays) when PcW is mutated whereas it is highly decreased (84%) when P<sub>A</sub> is mutated.**



**FEMS-1915**

**Antimicrobial resistance**

**IN-VITRO EVALUATION OF NISIN ALONE AND IN COMBINATION WITH POLYCATIONIC POLYMERS AGAINST HOSPITAL-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (HA-MRSA) STRAINS**

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**Background**

Nasal colonization by Methicillin-resistant *Staphylococcus aureus* (MRSA) is a problem in the hospital setting due to the required hygienic measures as well as the possibility of developing an infection. Polycationics such as Nisin or Chitosan are known to possess antimicrobial activity against a whole set of different bacteria including *Staphylococcus aureus*. The use of combinations may be one way to increase antimicrobial activity and to reduce side effects at the same time.

**Objectives**

The aim of this study was to investigate whether the use of the antimicrobial lantibiotic Nisin in combination with different types of polyethylenimine and chitosan show an increased antimicrobial effect towards MRSA.

**Methods**

The tested MRSA strains were provided by the Institute of Medical Microbiology and Hygiene Regensburg, Germany. Minimum inhibitory concentration (MIC) values were determined according to the CLSI microdilution broth method and at pH 5.5 to assess the efficacy of the antimicrobials. Likewise checkerboard assays were performed at pH 5.5.

**Conclusions**

Combining the antimicrobial peptide Nisin with the polycationic polymers shows promising activity against MRSA strains. Although no synergism could be detected, additive effects were clearly visible. This may be of advantage for the formulation of drug delivery systems for the treatment of nasal MRSA infections.

**FEMS-2702**

**Antimicrobial resistance**

## **MOLECULAR DETECTION AND CHARACTERISATION OF METALLO-B-LACTAMASE GENES AND INTEGRONS OF IMIPENEM-RESISTANT PSEUDOMONAS AERUGINOSA**

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### **Background**

The increase in the prevalence of carbapenem-resistant *Pseudomonas aeruginosa* isolates, particularly caused by the metallo- $\beta$ -lactamases (MBL) is of great concern in the clinical settings worldwide,

### **Objectives**

Here, we report the detection and identification of MBL producing *P. aeruginosa* in our hospital (Istanbul University Medical Center, Turkey), and the characterization of MBL cassette-containing integrons in 90 imipenem-resistant *P. aeruginosa* isolates. Of the isolates investigated in this study, 32 (94%) MBL-positive strains were identified, harboring either *blaIMP* or *blaVIM* genes. The genes detected from MBL-positive isolates were *blaIMP-7*, *aacC1*; *blaVIM-11*; *aacA7*, *blaVIM-2*, *attI*, and *aacA7*.

### **Methods**

In this study, three phenotypic methods, i.e., combined IMP-EDTA disk test (CDT), double-disk synergy test (DDST), and IP/IPI *E*-test) were evaluated for MBL detection in imipenem-resistant *P.aeruginosa* (IRPA) clinical isolates, in comparison to PCR detection of MBL genes as the gold standard. Integrons and their associated gene cassettes were characterized and the genetic relatedness of the isolates was investigated using random amplification of polymorphic DNA (RAPD) analysis.

### **Conclusions**

This study highlights the resistance to imipenem due to IMP- and VIM-producing *P. aeruginosa* and their associated class 1 integrons. Horizontal dissemination of the class 1 integron-associated MBL genes may contribute to the further emergence of carbapenem resistance in other Gram-negative bacteria. Therefore, appropriate surveillance and control measures are essential to prevent the further spread of MBL-producing organisms in hospitals. Further studies should be carried out to give a

better understanding of the impact of integrons on the dissemination of antimicrobial resistance in the clinical settings.

## FEMS-0725

### Antimicrobial resistance

#### DETECTION OF AGR SYSTEM AND ANTIMICROBIAL RESISTANCE IN *S. EPIDERMIDIS* PRODUCER BIOFILM ISOLATED OF AN UNIVERSITY HOSPITAL OVER 20 YEARS.

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### Background

*S. epidermidis* isolated from blood culture is highly important in a hospital.

### Objectives

To establish the *agr* locus, the susceptibility of biofilm-producing *S. epidermidis* specimens to antimicrobial agents, and to analyze the clonal profile of *S. epidermidis* isolated from blood culture from an University Hospital, Brazil, along a 20-year period.

### Methods

Detection of biofilm-related genes (*icaA*, *icaB*, *icaC* and *icaD*, *bhp* and *aap*) by polymerase chain reaction (PCR) and expression by reverse transcriptase polymerase chain reaction (RT-PCR) showed 83.6% of *ica* operon, 11.5% of *bhp* gene, and 32.8% of *aap* gene for 61 *S. epidermidis* specimens. The *agrI* and *agrII* loci were detected in 77% and 19.7%, respectively. Oxacillin-resistance was verified by detection of *mecA* gene in 90.1%, and the minimal inhibitory concentration (MIC) values assessed by E-test have shown 83.6% resistance to oxacillin. Clonal profile determination showed 11 clusters, and the ST2 type was determined to be the major cluster.

### Conclusions

The ability of *S. epidermidis* to produce biofilm has been shown, with predominant *agrI* locus, oxacillin-resistance, and highest prevalence of SCC*mec* type III. Some isolates showed resistance to newer antimicrobial agents, and molecular type determination revealed clones persisting for up to 17 years. The major one was the *S. epidermidis* ST2 invasive clone, pointing to the relevance of considering such

organisms as important pathogens with potential ability to cause serious infection in inpatients, as well as taking into account the potential dissemination of such resistant pathogenic clones in hospital settings.

**FEMS-0748**

**Antimicrobial resistance**

**RESPONSE TO ENVELOPE STRESS BY THE ZRAP/SR SYSTEM IN  
ESCHERICHIA COLI**

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**Background**

To detect and respond to cell envelope damages, Gram-negative bacteria possess multiple envelope stress pathways. The *E. coli*, ZraPSR system is composed of a two component system ZraSR that comprises a membrane associated sensor kinase ZraS and a cytoplasmic response regulator ZraR. This system has the particularity to encompass a third component, the periplasmic protein ZraP. The *zraP* gene is up-regulated by ZraR in the presence of zinc. Moreover, the deletion of *zraP* in *Salmonella typhimurium* dramatically affected its resistance to polymyxin B, a cationic antimicrobial peptide (Appia-Ayme *et al*, 2012).

**Objectives**

A connexion might exist between Zn(II) metabolism and antibiotics sensitivity, mediated by the ZraPSR system. In this study, we investigated this connexion and the ZraPSR signaling pathway in *E. coli*.

**Methods**

WT and  $\Delta zraP$  strains were phenotypically tested for their drug susceptibility profiles.  $P_{zraP}::lux$  and  $P_{zraSR}::lux$  transcriptional fusions were monitored in the presence of metals and drugs. Minimal inhibitory concentrations of zinc, copper, lead and antibiotics were determined for  $\Delta zraP$ ,  $\Delta zraS$  and  $\Delta zraR$  *E. coli* strains.

**Conclusions**

At high zinc concentrations, the expression levels of the two divergent operons *zraP* and *zraSR* increased by 100- and 40- fold, respectively. This suggested that the ZraPSR system is involved in zinc homeostasis. However we showed that high metal concentrations do not affect the survival rate of any *zraP*, *zraS* or *zraR* mutants. In contrast, these *zraPSR* mutants were more sensitive to some envelope targeting antibiotics. This indicates that ZraPSR contributes to the envelope stress response of *E. coli*.

## FEMS-2772

### Antimicrobial resistance

#### IDENTIFICATION OF DIVERSE CAPSULAR TYPES IN MULTIDRUG-RESISTANT (MDR) KLEBSIELLA PNEUMONIAE CLONES USING WZI SEQUENCING AND FTIR

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#### Background

*Klebsiella pneumoniae* polysaccharide capsule is a well-known determinant of virulence and pathogenicity, but their identification by conventional methods is laborious and time-consuming. A genotypic-based method has been proposed for capsular typing based on *wzi* sequencing, one of the genes from the *cps* cluster. Identification of capsular types in MDR *K. pneumoniae* high-risk clonal groups suggested intraclonal diversity but it has mainly been explored for carbapenemase-producing CG258 isolates.

#### Objectives

To assess inter and intraclonal diversity of capsular types in a representative collection of MDR *K. pneumoniae* isolates by genotypic, phylogenetic and spectroscopic approaches.

#### Methods

One hundred and four previously characterized MDR *K. pneumoniae* isolates from different clones or CG (ST14, ST15, ST39, ST54, ST101, ST147, ST336, CG258) producing diverse ESBLs and/or carbapenemases were studied. They represent a snapshot of MDR clones responsible for outbreaks or endemic situations in different countries (Brasil, Greece, Poland, Portugal, Romania, Spain). Characterization of capsular types included sequencing and phylogenetic analysis of *wzi* genes (including *wzi* sequences from genomes deposited in NCBI), and Fourier Transform Infrared Spectroscopy (FTIR) coupled with multivariate data analysis (PCA/PLSDA).

#### Conclusions

The diversity of capsular types identified among CG15, CG258 or ST11 isolates highlights an intraclonal diversity that might reflect a suboptimal resolution of MLST-based typing. The clustering of the isolates based on multivariate data analysis of

FTIR spectra according to sequence types and capsular types, which was corroborated by the topology obtained from the phylogenetic tree of *wzi* sequences, suggests the potential application of FTIR for discrimination of *K. pneumoniae* isolates.



**FEMS-3068**

**Antimicrobial resistance**

## **EFFICACY OF BIOACTIVE COMPOUNDS FROM ACACIA ARABICA AGAINST MULTI-DRUG RESISTANT CLINICAL BACTERIAL PATHOGENS**

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### **Background**

The emergence of infectious diseases and development of drug resistance in pathogenic bacteria at an alarming rate is a matter of serious concern. Therefore, there is an urgent need to discover novel strategies and identify new antimicrobials from natural substances for development of next generation drugs. Hence screening of novel antibacterial agents was carried out from the bark of *Acacia arabica* with traditional value in ayurvedic medicine.

### **Objectives**

The study aimed to identify bioactive compounds with antibacterial activity isolated from bark extracts of *Acacia arabica*. The inhibitory and cidal concentration of the compounds were evaluated. Further, synergistic effect of potent bioactive compounds with standard antibiotic was determined.

### **Methods**

Bark extracts of *Acacia arabica* were prepared by cold percolation method using polar to non-polar solvents in succession. Antimicrobial activity of the extracts was evaluated using Agar well diffusion method against multi-drug resistant bacteria. Using chromatographic techniques, the bioactive compounds from the extracts were isolated and purified. The purity of the compounds was assessed using high performance liquid chromatography and the compounds were identified using GCMS and NMR spectroscopic techniques. The inhibitory and cidal concentration of the compounds were determined using Resazurin micro-titre assay plate method. The compound exhibiting maximum antibacterial activity with a minimum MIC value was evaluated for synergism with antibiotic.

### **Conclusions**

The results showed that acetone extract was most effective in inhibiting the multi-drug resistant bacterial pathogens. The compounds identified from acetone extract showed positive synergism with antibiotic tested suggesting the possibility of new drug development.

## **FEMS-2361**

### **Antimicrobial resistance**

#### **NANO-ENCAPSULATED COLISTIN SULPHATE: ANTIPSEUDOMONAL ACTIVITY**

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### **Background**

*Pseudomonas aeruginosa* infects the respiratory tract of cystic fibrosis (CF) patients. Multidrug resistant phenotypes and a high capacity to form stable biofilms are frequent. Despite colistin-resistance is infrequent, often emerges in patients treated with inhaled formulations for long periods. The use of nanoparticles can contribute to overcome drug resistance mechanisms. Some authors have reported that lipid nanoparticles loaded with aminoglycosides are more effective than free formulations against clinical isolates.

### **Objectives**

The aim was to explore antimicrobial activity of nanoencapsulated colistin (SLN-NLC) versus free drug against *P.aeruginosa* clinical isolates from CF patients and to investigate their efficacy in biofilm eradication.

### **Methods**

10 mg of colistin was mixed with a 5% (w/v) Precirol® ATO 5 dichloromethane solution. Then, the organic phase and Poloxamer 188 at 1% w/v and Polysorbate 80 at 1%w/v were mixed and emulsified by sonication, solvent evaporated and SLNs obtained filtered. NLC were made with Precirol® ATO 5 and Miglyol® 812 as lipid core. Susceptibility of planktonic bacteria to antimicrobials was examined by using the broth microdilution method and time-kill kinetic curves. Minimal Biofilm Eradication Concentration (MBEC) was determined to assess antimicrobial susceptibility of sessile bacteria. Treated and untreated biofilms were visualized by Atomic Force Microscopy (AFM).

### **Conclusions**

Colistin nanoparticles presented slightly higher activity than free drug against planktonic bacteria. On the contrary, nanoencapsulated colistin was much more efficient in the eradication of biofilms than free colistin. Thus, these formulations have to be considered as a good alternative therapeutic option to treat *P. aeruginosa* infections.

## FEMS-1965

### Antimicrobial resistance

#### CANDIDA GLABRATA PHENOTYPIC SWITCHING AND ITS ASSOCIATION WITH ECHINOCANDIN ANTIFUNGAL RESISTANCE.

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#### Background

*Candida glabrata* stands up as the second most prevalent pathogen of mucosal and systemic fungal infection, being the non-*albicans* species with the highest mortality rate associated. Since it possesses both innate and acquired resistance against azole antifungal drugs, echinocandins became the first-line therapy in many hospitals. Recently, *C. glabrata* pathogenicity was associated with phenotypic switching, an important virulence attribute in *C. albicans*, but yet poorly characterized in *C. glabrata*.

#### Objectives

We intend to reveal phenotypic switching as a predictor to uncover the emergence of echinocandins antifungal resistance in *C. glabrata*.

#### Methods

Echinocandin resistance induction assays were performed with CBS 138 strain and two clinical isolates. *C. glabrata* strains were daily exposed to the following echinocandins: caspofungin (CSF), micafungin (MCF) and anidulafungin (ANF). Every 5 days of incubation, minimal inhibitory concentrations (MIC) were determined, according to the CLSI protocol M27-A3 S4.

Phenotypic switching assessment was carried out during induction of antifungal resistance, by plating each strain in medium containing phloxine B.

#### Conclusions

At the beginning of induction assays susceptible colonies were Light Pink (LP) or Medium Pink (MP). In all strains, resistance to echinocandins was detected at day 5 and colonies were MP and Dark Pink (DP). The MICs of the different phenotypes were determined and differences in the susceptibility to echinocandins were found: LP and MP colonies remain susceptible and the DP were resistant.

The continuous exposure to serum levels of echinocandins promotes the acquisition of antifungal resistance in *C. glabrata* together with the emerging of phenotypic switching.

## FEMS-1912

### Antimicrobial resistance

#### HIGH VIRULENT CMY-2 AND CTX-M-2-TYPE-PRODUCING *E. COLI* CAUSING COLIBACILLOSIS IN COMMERCIAL TURKEYS FROM BRAZIL

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#### Background

A high diversity of AmpC and extended-spectrum (ESBL) beta-lactamases has been described in Brazil among isolates from nosocomial and community settings, but this is the first report of these enzymes in turkey Brazilian farms.

#### Objectives

The aim of this study was to characterize ESBL and plasmid-mediated AmpC-producing *E. coli* isolated from commercial turkey in Brazil.

#### Methods

In 2009, 227 *E. coli* strains were isolated from air sac of turkeys condemned by airsacculitis and screened for ESBL and pAmpC production. MICs were determined by microdilution, and beta-lactamases-encoding genes and virulence factors were investigated by PCR. Plasmids were extracted and transformed into Top10 electrocompetent *E. coli* strain (Invitrogen<sup>TM</sup>) followed by direct sequencing. Genetic relatedness of beta-lactamase-positive strains was determined by ERIC-PCR fingerprinting.

#### Conclusions

The *bla*<sub>CTX-M-2</sub> and *bla*<sub>CMY-2</sub> genes were confirmed in four and seven APEC strains, respectively; which were clonally unrelated, showing high MIC to ceftiofur (MIC<sub>50</sub>≥64 mg/L). The *bla*<sub>CMY-2</sub> genotype was associated with the presence of an IncI1 plasmid of approximately 94kb, which showed 98% of similarity with pSTM709 plasmid (Genbank: HG428759.1), identified in *Salmonella* Typhimurium in Uruguay, confirming its spread in Latin America. Much more concerning is the high virulent profile exhibited by these beta-lactamase-producing APEC, which shared the same virulence factors of human ExPEC. In this regard, the dissemination of cephalosporin-resistant bacteria carrying *bla*<sub>ESBL</sub> and/or *bla*<sub>CMY-2</sub> genotypes has the potential to impact both veterinary and human therapeutic treatment options, which requires more surveillance of antimicrobial resistance in bacteria from food-producing animals.

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## **FEMS-0773**

### **Antimicrobial resistance**

#### **TESTING THE MUTANT SELECTION WINDOW (MSW) HYPOTHESIS WITH GRAM-NEGATIVE BACTERIA EXPOSED TO THE FLUOROQUINOLONES IN IN VITRO DYNAMIC MODELS**

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### **Background**

According to the MSW hypothesis, resistant mutants are selected at antibiotic concentrations between the MIC and the mutant prevention concentration (MPC). This hypothesis has been tested mainly with fluoroquinolone-exposed Gram-positive pathogens but not Gram-negatives.

### **Objectives**

To test the MSW hypothesis with *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, ciprofloxacin pharmacokinetics were simulated in an *in vitro* dynamic model.

### **Methods**

Four strains each of *E. coli* and *Pseudomonas aeruginosa* and three strains of *K. pneumoniae* with MPC/MIC ratio from 4 to 64 were exposed to twice-daily ciprofloxacin for three days over 50-380-fold ranges of the ratio of the area under the concentration-time curve (AUC) to the MIC. Peak antibiotic concentrations were simulated to be close to the MIC, between the MIC and the MPC, and above the MPC.

### **Conclusions**

With all three bacterial species, resistant mutants were enriched at ciprofloxacin concentrations that fell into the MSW for most of the dosing interval but not at concentrations out of the MSW. Both AUC/MIC- and AUC/MPC-resistance relationships were bell-shaped, showing applicability of the MSW hypothesis. These relationships predict highly variable “anti-mutant” AUC/MIC ratios - 525-1070 h (*E. coli*), 560-1800 h (*K. pneumoniae*) and 225-1100 h (*P. aeruginosa*) – and AUC/MPC ratios – 25-100, 28-140 and 7-204 h, respectively.



**FEMS-0775**

**Antimicrobial resistance**

**BACTERIAL RESISTANCE STUDIES USING IN VITRO DYNAMIC MODELS: NEW INSIGHT IN THE DESIGN OF “ANTI-MUTANT” ANTIBIOTIC DOSING**

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**Background**

*In vitro* models that simulate human pharmacokinetics of antibiotics allow pharmacodynamic comparisons. This approach can be used to examine the abilities of antimicrobials to suppress not only antibiotic-susceptible pathogens but also their resistant mutants.

**Objectives**

To assess the ability of fluoroquinolones, lipo- and glycopeptides to prevent the selection and/or to suppress resistant mutants of *Staphylococcus aureus*, multiple dosing regimens of the antibiotics were simulated in an *in vitro* dynamic model.

**Methods**

A series of monoexponential pharmacokinetic profiles that mimic once-daily administration of moxifloxacin, gatifloxacin, levofloxacin and daptomycin and twice-daily dosing of ciprofloxacin and vancomycin, were simulated with half-lives reported in humans. The simulated ratios of the area under the concentration – time curve (AUC) to the MIC varied in a wide range that includes clinically relevant AUC/MICs.

**Conclusions**

With each agent resistant *S. aureus* mutants were enriched in a concentration-dependent manner. This enrichment was accompanied by concomitant loss in the susceptibility of antibiotic-exposed bacteria. Both in terms of the population data analysis and susceptibility testing, AUC/MIC-resistance relationships were bell-shaped. Based on these relationships, the threshold “anti-mutant” AUC/MIC ratios were predicted. With moxifloxacin and gatifloxacin but not levofloxacin and ciprofloxacin, and with daptomycin and vancomycin, the predicted thresholds were within the clinically attainable AUC/MIC ratios.

**FEMS-0893**

**Antimicrobial resistance**

**CHARACTERIZATION OF MUTATION IN STREPTOMYCIN-RESISTANT MYCOBACTERIUM TUBERCULOSIS AND THE ASSOCIATION BETWEEN BEIJING-LINEAGE AND DUAL-MUTATION IN GIDB GENE**

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**Background**

In developing countries, resistance of *Mycobacterium tuberculosis* to streptomycin (STR) is a severe threat to tuberculosis treatment. Mutations in *rpsl*, *rrs*, and *gidB* genes are reported to be related to STR resistance, some of which are suggested to be associated with *M. tuberculosis* lineage, while this association is geographically varied.

**Objectives**

In this study, we aimed to investigate the mutation characteristics of STR resistance and the association between the polymorphism of drug-resistant genes and *M. tuberculosis* lineage in Sichuan, China.

**Methods**

A total of 180 STR-resistant and 47 susceptible *M. tuberculosis* clinical isolates were genotyped by spoligotyping followed by mutation sequencing of *rpsl*, *rrs* and *gidB* genes.

**Conclusions**

No mutation was released in *rpsl* and *rrs* genes among sensitive isolates, while in resistant ones, 150 harbored mutations in *rpsl* or *rrs*, suggesting a correlation between STR-resistance and mutations of these two genes. Mutation K43R in *rpsl* was found to strongly associate with high-level STR resistance ( $P < 0.01$ ), while those in *rrs* and *gidB* potentially contributed to low-level resistance ( $P < 0.05$ ). No significant geneal association was presented between STR-resistance and Beijing genotype, however, Beijing genotype was significantly correlated with high-level STR-resistance, as well as with K43R ( $P < 0.01$ ), indicating that Beijing genotype has an evolutionary advantage under streptomycin pressure. Interestingly, a dual mutation of E92D and A205A in the *gidB* gene was detected in all isolates of Beijing genotype, suggesting this dual mutation have the potential to be used as an effective marker for identifying Beijing genotype.

**FEMS-1683**

**Antimicrobial resistance**

## **STRAIN DEPENDENT RESPONSE OF CANDIDA ALBICANS ATCC AND CLINICAL STRAINS TO PHOTODYNAMIC INACTIVATION**

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### **Background**

Yeast infections, caused by *Candida albicans* are health- and life-threatening, especially to patients with chronic diseases or immunodeficiency. Photodynamic inactivation (PDI) can be successfully used to overcome the problem of resistance of microorganisms to classical antimicrobial chemotherapies.

### **Objectives**

The aim of this study was to compare the effect of nine imidazoacridinone (IA) derivatives used as photosensitizing agents in PDI against *C. albicans* ATCC and clinical strains.

### **Methods**

The Quality Control and clinical strains of *Candida albicans* suspensions were treated with IA derivatives (50  $\mu$ M concentration) and irradiated with 50 and 100 J/cm<sup>2</sup>. The light source used was custom made LED lamp (Secure Media Poland), of maximum output power of 630 mW/cm<sup>2</sup>, which emits blue light (405 nm). Cell suspensions kept in the dark were used as controls. After irradiation serial dilutions from 10<sup>-1</sup> to 10<sup>-4</sup> were obtained from every sample and colony forming units/ml (CFU) were determined. Moreover, the accumulation of IAs in 3 ATCC strains was measured using spectrophotometric methods.

### **Conclusions**

The use of IAs in PDI was able to promote a significant antimicrobial effect against the planktonic forms of *C. albicans* (>5 log<sub>10</sub> units reduction in survival). The PDI effect did not exclusively depend on the chemical structure of the photosensitizing compounds or their accumulation in *C. albicans* cells. We could observe very strong strain-dependent response to PDI treatment. Our results showed that PDI outcome did not strictly depend on physico-chemical processes, but biological background of particular strain played also an important role in this reaction.

**FEMS-1987**

**Antimicrobial resistance**

**MOLECULAR CHARACTERIZATION AND ANTIBIOTIC SUSCEPTIBILITY OF METICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) IN NORTH INDIA, WITH SPECIAL REFERENCE TO COMMUNITY ACQUIRED MRSA**

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**Background**

Infiltration of community acquired meticillin resistant *Staphylococcus aureus* (CA-MRSA) strains into hospitals and hospital acquired (HA) MRSA into community has become a major hurdle leading to poor clinical outcomes. Role of Panton-Valentine leukocidin (PVL) and arginine catabolic mobile element (ACME), previously associated with CA-MRSA, still remains controversial.

**Objectives**

To study relative frequency of CA and HA- MRSA in outpatients, inpatients and to find and correlate presence of PVL and ACME.

**Methods**

Total 147 MRSA isolates were collected from clinical bacteriology lab of a tertiary care hospital. Isolates were characterized by PCR amplification of *mecA*, PVL, ACME genes and staphylococcal cassette chromosome *mec* (SCC*mec*) typing. Patient risk factors, antimicrobial susceptibility profiles and pcr results were compared. A total of 129 MRSA strains were *mecA* positive, and 34 (27.9%) were PVL gene positive. SCC*mec* III were 31 (24%), 41 were SCC*mec* IV (31.78%), and 32 were SCC*mec* V (24.8%). Of all SCC*mec*III strains, multidrug resistant(MDR) - 70%. In SCC*mec* IV, 51.2% were MDR and in *mec*V strains - 31.25% MDR, further supported by presence of risk factors. PVL positivity in case of *mec*III,IV,V was 2.94%,52.9%,41% respectively. ACME *arc*gene was found in 5(3.87%) cases, 2 of 5 were CA-MRSA non MDR isolates without risk factors. ACME *OPP3*gene was absent.

**Conclusions**

This study demonstrated the presence of CA-MRSA in hospital settings and vice versa. *mec*III MDR strains are no longer restricted to hospital settings and *mec*IV, V are frequently found in hospital settings with MDR characteristics. But PVL is still present mostly in *mec*IV and V.

**FEMS-2329**

**Antimicrobial resistance**

## **CARBAPENEMASE-PRODUCING KLEBSIELLA PNEUMONIAE AND ESCHERICHIA COLI IN SERBIA**

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### **Background**

Emergence and dissemination of carbapenemase-producing *Enterobacteriaceae* is rising worldwide, representing a public health threat. Although most European countries have already established their national surveillance programs considering carbapenemase-producing enterobacteria, so far there are no reports from Serbia.

### **Objectives**

The aim of the study was to determine the occurrence and types of carbapenemases in carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in Serbia.

### **Methods**

Strains of *Klebsiella pneumoniae* and *Escherichia coli* from 14 different hospitals resistant to at least one carbapenem (imipenem, meropenem, ertapenem) were collected from November 2013 to May 2014. In National Reference Laboratory for Susceptibility Testing carbapenem resistance was confirmed. PCR assays were performed for the detection of genes encoding carbapenemases (*bla*<sub>OXA-48</sub>, *bla*<sub>VIM</sub>, *bla*<sub>NDM</sub> and *bla*<sub>KPC</sub>).

### **Conclusions**

Of 125 collected strains 117 (93.6%) were *Klebsiella pneumoniae* and 8 (6.4%) were *Escherichia coli*. Sixty seven (53.6%) strains were obtained from urine, 25 (20%) from blood, 19 (15.2%) from wound secretions and 14 (11.2%) from lower respiratory tract secretions. Seven (87.5%) *Escherichia coli* harbored *bla*<sub>NDM</sub> gene and in one isolate no carbapenemase encoding genes were detected. Gene *bla*<sub>NDM</sub> was found in 34 (29.1%) *Klebsiella pneumoniae*, *bla*<sub>OXA-48</sub> in 10 (8.5%), *bla*<sub>KPC</sub> in 1 (0.9%), and 7 (6%) strains harbored both *bla*<sub>NDM</sub> and *bla*<sub>OXA-48</sub>, while in 65 (55.6%) *Klebsiella pneumoniae* no carbapenemase encoding genes were detected.

In Serbia the most common type of carbapenemase in both carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* is NDM. Carbapenemase-

producing *Klebsiella pneumoniae* and *Escherichia coli* were most frequently isolated from urine.

**FEMS-0842**

**Antimicrobial resistance**

## **MONITORING OF THE ENTEROCOCCI RESISTANCE MECHANISMS TO AMINOGLYCOSIDES**

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### **Background**

*Enterococcus faecalis* and *Enterococcus faecium* are opportunistic bacteria responsible for frequent nosocomial infections. Presently, resistance of enterococci to antibiotics, especially aminoglycosides, glycopeptides and oxazolidinones is becoming a severe clinical problem.

### **Objectives**

The aims of the study were as follows: determination of phenotypic patterns of enterococci resistant to aminoglycosides, detection of genes responsible for above resistance, investigation of relationships between analyzed isolates.

### **Methods**

125 *E. faecalis* and 49 *E. faecium* isolates resistant to aminoglycosides, obtained from clinical materials in one city hospital during period 2009-2012, were investigated. Besides estimation of antibiotic susceptibility patterns, the search for HLAR (gentamicin MIC > 128mg/L and streptomycin MIC > 1024mg/L), HLGR (gentamicin MIC > 128mg/L) and HLKR (kanamycin and amikacin MICs > 512mg/L) phenotypes was undertaken. The presence of the following genes: *aac(6')-Ie-aph(2'')-Ia*, *aph(2'')-Ib*, *aph(2'')-Ic*, *aph(2'')-Id*, *aph(3'')-IIIa*, *ant(4')-Ia*, *ant(3'')-Ia*, *ant(3'')-Ia*, responsible for resistance to aminoglycosides was detected by PCR technique. Clonal distribution and relationship between isolates was investigated by PFGE after bacterial DNA digestion with *Sma*I restrictase.

### **Conclusions**

80% of the analyzed isolates belonged to the HLAR group, remaining isolates possessed phenotype HLGR. HSLR phenotype was not detected. Genetic analysis showed that *aac(6')-Ie-aph(2'')-Ia* gene responsible for the high resistance to

gentamicin and for cross-resistance to other aminoglycosides (except streptomycin) is the most frequently presented gene among the isolates of tested enterococci. The next common gene was *aph(3'')-IIIa*. Restriction genomic DNA fragments analysis showed large genetic diversity of tested isolates.



**FEMS-2993**

**Antimicrobial resistance**

## **ANTIMICROBIAL RESISTANCE OF CLINICAL SALMONELLA STRAINS IN EDIRNE, TURKEY: FOUR YEAR TREND**

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### **Background**

Salmonella is one of the most common pathogens of the gastrointestinal tract for both humans and animals leads to food-borne outbreaks and various infections. Accurate diagnosis and treatment of this important pathogen is only achieved by knowing its serogroup and antibiotic susceptibility.

### **Objectives**

In our study, we tried to reveal the distribution and antibiotic resistance of Salmonella strains in the community, also examined the effectiveness of multiplex polymerase chain reaction in the identification of Salmonella.

### **Methods**

Bacterial identification and antibiotic susceptibility tests of 105 Salmonella strains, isolated from the clinical samples (between 2009-2013), were conducted with VITEK2 (Biomérieux, France) automatized system. Strains were grouped with both slide agglutination by using Salmonella polyvalent and group specific antisera (Plasmatec, UK) and multiplex PCR. Multiplex PCR was performed by using six sets of primers targeting O-antigen synthesising gene regions in A, B, C1, D and E serogroups commonly found in clinical isolates.

### **Conclusions**

O-grouping results revealed that serogroup D(%68) and C1(%23) are the most common causes of Salmonella oriented diarrhea in Edirne, respectively. Multiplex PCR results showed 100% compatibility with serologic diagnosis. The highest level of resistance found against to ampicillin(%16) among all antibiotics. Among four years there is an increasing resistance to cephalosporins, trimetoprim–sulfamethoxazol and fluoroquinolones.

Salmonella serogroup D is the most frequent serogroup isolated in Edirne and emerging resistance to several antibiotics might be a serious health problem in the future. According to our study, also, multiplex PCR is a reliable and reproducible method in O-grouping of Salmonella.

**FEMS-2926**

**Antimicrobial resistance**

## **THE ROLE OF REACTIVE OXYGEN SPECIES IN ANTIBIOTIC INDUCED CELL DEATH IN BURKHOLDERIA CEPACIA COMPLEX BACTERIA**

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### **Background**

It was recently proposed that bactericidal antibiotics kill bacteria through a common mechanism involving the production of reactive oxygen species (ROS). However, this mechanisms involving the production of hydroxyl radicals has become the subject of debate. Since the contribution of ROS to antibiotic mediated killing most likely depends on the conditions, differences in experimental procedures are expected to be at the basis of the conflicting results reported in the literature.

### **Objectives**

The goal of this study was to compare methods used to measure the production of ROS and to study ROS production in *Burkholderia cepacia* complex (*Bcc*) biofilms and planktonic cultures.

### **Methods**

Both direct (ROS-specific stainings) and indirect methods (gene-expression analyses, genetic and phenotypic experiments, detection of protein carbonylation and DNA oxidation) to measure ROS were tested and antibiotics belonging to different classes were included.

### **Conclusions**

While some methods were not sensitive enough, others were found to be useful only in some conditions. Since fluorescein based stainings were shown to be pH sensitive and most *Bcc* species, due to resistance need to be treated with antibiotics in high concentrations (which has a marked influence on pH), these dyes cannot be added simultaneously with the antibiotics and control solutions should have the same pH as the antibiotic solutions tested.

Overall our results highlight some methodological key issues to be considered when evaluating the contribution of ROS in antibiotic mediated killing and although not one ideal method could be identified the different methods suggest that ROS is involved in antibiotic mediated killing in *Bcc* species.



**FEMS-1506**

**Antimicrobial resistance**

## **COLISTIN-RESISTANT MECHANISMS OF KLEBSIELLA PNEUMONIAE IN TAIWAN**

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### **Background**

Colistin is an important antibiotic for the treatment of carbapenem-resistant *Klebsiella pneumoniae* infection.

### **Objectives**

The colistin resistance mechanism(s) of 26 colistin-resistant *K. pneumoniae* strains in Taiwan were studied.

### **Methods**

We detected *pmrH* mRNA expression by RT-qPCR and analyzed sequences of *pmrHFIJKLM* regulators (MgrB, PhoPQ, PmrAB and PmrD).

### **Conclusions**

Determination of the capsular types and multilocus sequence types (MLSTs) of colistin-resistant strains showed that K64 and ST11 were the predominant capsular type (50%) and sequence type (53.9%), respectively. The expression of *pmrHFIJKLM* operons in 25/26 (96.2%) colistin-resistant strains was significantly higher than in colistin-sensitive strains. The promoter and coding regions of *mgrB* were interrupted by a transposon in 5 and 3 strains, respectively, and *mgrB* was absent in 2 strains. Moreover, MgrB Cys28Tyr and Stop48Tyr mutations were observed in 1 and 3 strains, respectively. Site-directed mutagenesis revealed that MgrB Stop48Tyr and PhoQ Leu26Pro mutations in the colistin-sensitive strain NTUH-K2044 increased the mRNA expression of *pmrH* by 5.3-fold and 39.6-fold, respectively, and both mutations increased the NTUH-K2044 minimal inhibitory concentration for colistin by 32-fold. These results suggest that capsule type K64 is the prevalent type in colistin-resistant strains, and interruptions in *mgrB* which increased expression of the *pmrHFIJKLM* operon are major mechanisms contributing to colistin resistance in Taiwan. In addition, novel single amino acid changes in MgrB (Stop48Tyr) and PhoQ (Leu26Pro) were observed to contribute to colistin resistance.

**FEMS-1610**

**Antimicrobial resistance**

**THE NEW MLS<sub>B</sub> RESISTANCE GENE ERM(45) IS LOCATED ON A GENOMIC ISLAND IN STAPHYLOCOCCUS FLEURETTII**

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**Background**

***Staphylococcus fleurettii* is a commensal of animals which can occasionally cause bovine mastitis. *S. fleurettii* strain JW205 isolated from bovine milk in Switzerland exhibited inducible resistance to macrolide, lincosamide and streptogramin B (MLS<sub>B</sub>) antibiotics suggesting the acquisition of an erythromycin resistance methylase gene (*erm*), but no known Erm determinant was detected.**

**Objectives**

To identify the MLS<sub>B</sub> resistance mechanism of *S. fleurettii* JW205 and determine if it is located on a mobile genetic element.

**Methods**

Whole genome sequencing of strain JW205 and MLS<sub>B</sub>-susceptible strain M404 (Ion Torrent, Illumina); Comparative analysis of both genomes (progressiveMauve); Cloning and expression of a putative MLS<sub>B</sub> resistance gene in *Staphylococcus aureus* RN4220 to demonstrate functionality and inducible expression; Transformation assays by conjugation and electroporation.

**Conclusions**

The new MLS<sub>B</sub> resistance gene *erm*(45) showed 64% aa identity with the nearest related known methylase Erm(B). It was identified on an 11,513-bp novel genomic island in *S. fleurettii* JW205 (ENA acc. no. LN680996). When cloned into *S. aureus*, *erm*(45) confers inducible resistance to macrolide, lincosamide and streptogramin B. Although a circular form of the island was observed, the element could not be transferred into *S. aureus*. Detection of this new methylase emphasized the role of *S. fleurettii* as reservoir of antibiotic resistance genes and demonstrated once again the importance of animal bacteria as pool for novel genetic elements.

**FEMS-2985**

**Antimicrobial resistance**

## **RESEARCHANT HUB: A COMPREHENSIVE LITERATURE-BASED ANTIMICROBIAL SUSCEPTIBILITY DATABASE**

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### **Background**

Antimicrobial resistance is an ever-increasing global public health treat. This phenomenon has stimulated a vigorous research on the field of the antimicrobial susceptibilities and new antimicrobials for the treatment of the so-called super-bugs. The decline in the efficacy of the traditionally used antimicrobials is accompanied by the increasing focus on less conventional antimicrobials agents such as plant extracts and experimental compounds. Scientific literature is a rich source containing thousands of either traditionally overlooked or recently discovered antimicrobial compounds. Unfortunately, the potential of these compounds is often not fully exploited. One reason is that literature information is scattered over various sources making the global assessments of the spectra or modes of the action of the compounds difficult.

### **Objectives**

To alleviate the problem of the lack of a comprehensive and centralized antimicrobial susceptibility database, we developed ResearchAnt Hub. RAH is a manually curated database that contains a collection of literature reported antimicrobial susceptibility data. Our compound library contains thousands of experimental compounds with reported antimicrobial activity that hold promise to become the next-generation antimicrobials.

### **Methods**

The antimicrobial susceptibility data was extracted from relevant peer-reviewed scientific journals by manual search and automatic mapping. The taxonomy of the organisms and physical properties of the known compounds were mapped against NCBI and PubChem, respectively.

### **Conclusions**

RAH is the largest antimicrobial susceptibility database to date. It is a tool that can be used for sharing information, search, prediction and discovery of the next-generation antimicrobial agents.

**FEMS-1077**

**Emerging diseases**

**THE INCIDENCE OF TOXIGENIC CLOSTRIDIUM DIFFICILE ASSOCIATED DIARRHEA IN MEXICAN CHILDREN AND ADULTS HOSPITALIZED.**

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**Background.** Toxigenic *Clostridium difficile* is the most common cause of nosocomial diarrhea, and it is usually a consequence of antibiotic treatment. The incidence and mortality rate of *C. difficile* infection have increased remarkably worldwide during the last two decades. In Mexico, *C. difficile* was not considered an important pathogen in children or adults with nosocomial diarrhea. At this time we ignore the incidence and characteristics of the infecting strains.

**Objective.** To study the epidemiology and virulence of *C. difficile* from Mexican children and adults with nosocomial diarrhea.

**Material and methods.** Stools samples were collected from adults and children with nosocomial diarrhea in two tertiary level hospitals in Mexico City. Stools were cultured in cycloserine cefoxitin fructose agar (CCFA), the *C. difficile* toxin B was confirmed by neutralization with antitoxin, and PCR was performed to identify *tcdA*, *tcdB*, *cdtA*, and *cdtB*.

**Results.** We identified *C. difficile* and its cytotoxic activity in 21 (84%) feces from children and 85 (68.5%) from adults. The majority of the fecal supernatants were positive for toxins of *C. difficile*, and strains were positive for *tcdA*, *tcdB*, *cdtA* and *B*. At present, toxigenic *C. difficile* is a common hospitals-acquired infection in Mexico City.

**FEMS-1241**

**Emerging diseases**

**ONE DECADE INVESTIGATION OF RIFT VALLEY FEVER (RVF) IN IRAN**

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**Background**

*Rift Valley Fever Virus (RVFV) is a member of Phlebovirus genus, Bunyaviridea family that cause acute zoonotic viral disease which mostly affect animals (livestock and specially sheep) but can also be a human threat.*

**Objectives**

This disease becomes a concern in Iran after outbreaks in Saudi Arabia and Yemen in 2000s.

**Methods**

In the last decade human and livestock sera have been screened; 1206 ovine, 405 caprine, 325 bovine and 28 camel samples and 37 suspected human sera were tested for RVFV in nine provinces in Iran (Sistan va Baloochestan, Khorasan, Tehran, Isfahan, Fars, Kerman, Kurdistan, Hormozgan and Booshehr).

**Conclusions**

None of these samples were IgG positive. Moreover, among 37 clinically suspected human cases with RVF symptoms, none of these samples were positive for RVFV. Despite no positive cases have been discovered until now, but as Iran is in a high risk region for RVF, the Laboratory of Arboviruses and Viral Hemorrhagic Fevers (National Ref Lab), Pasteur Institute of Iran, continues the surveillance of this acute hemorrhagic fever disease in Iran (in livestock and suspected human).



## FEMS-2942

### Emerging diseases

#### PATHOGENIC CLOSTRIDIUM SPP. IN BIOGAS PROCESSES

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#### Background

Several *Clostridium* spp. strains cause serious, occasionally lethal illness in humans and animals and can be introduced into biogas plants by contaminated feedstock.

#### Objectives

In order to enlarge the knowledge on the existence and behavior of these pathogens in biogas plants, this study focused (I) on the incidence of Botulinum neurotoxin (BoNT-)producing *Clostridium* spp., *C. difficile*, *C. novyi*, *C. haemolyticum*, *C. septicum* and *C. chauvoei* in process chains of full-scale biogas plants (screening) and (II) on the quantitative development of *C. botulinum* in lab-scale biogas digesters (sentinel chamber experiments).

#### Methods

The pathogens were detected by methods combining cultural enrichment with biomolecular analysis using quantitative Real-time PCR. For the screening, 154 samples were analyzed containing 29% plant substrates (e.g. silages), 11% animal substrates (e.g. manure), 27% main digester slurries and 33% digestion products (second stage digesters, storage tanks).

#### Conclusions

Pathogenic *Clostridium* spp. were not detected in any of the samples, except for *C. novyi* (4% of the samples contaminated) and *C. difficile*, which was found in approx. 50% of the samples, predominantly in animal substrates, digester slurries and digestion products. Sentinel chamber experiments resulted in reduction of *C. botulinum* by 1.3 to 2.8 log<sub>10</sub> units after 63 d (95.4% to 99.8% inactivation, D-value: 34.6 ± 11.2 d) at 38°C and by at least 99.7% already after 3 d (D-value: 1.0 ± 0.2 d) at 55°C, proving that the sanitary quality of the digestate compared to untreated manure concerning *C. botulinum* is improved by mesophilic and thermophilic biogas processes.

**FEMS-1949**  
**Emerging diseases**

**CASE REPORT: FIRST REPORT OF PULMONARY INFECTION CAUSED BY HORMOGRAPHIELLA ASPERGILLATA IN ITALY.**

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**Background**

*Hormographiella aspergillata* is a basidiomycete and belongs to the *Psathyrellaceae* family. It is an anamorph of *Coprinopsis cinerea*, which is normally found in compost and sewage. It has been rarely found in human infections, namely a few cases of pulmonary infections and isolated cases of endophthalmitis, endocarditis and sinusitis have been described, mainly in patients with haematological malignancies.

**Objectives**

We report on a case of pulmonary infection caused by *H.aspergillata* in a 26 years old male patient affected by “mixed phenotype acute leukemia” admitted to the University Hospital G.B.Rossi in Verona, Italy. This is the first case of infection caused by this pathogen reported in Italy.

**Methods**

Despite antifungal prophylaxis during hospitalization, Beta-D-Glucan assay resulted in increasingly positive values, followed by a positive result with the Galactomannan assay. Since the patient suddenly complained a severe pain in scapular region, thoracic RX and CT were performed. The CT revealed the presence of a dense mass compatible with a presumptive Aspergillosis in the right lung. After thoracotomy, a proper diagnosis of *H. aspergillata* was reached by morphological analysis of the growth on Sabouraux agar from biopsy specimen and identification of fungal pathogen was confirmed by molecular tools: sequencing and alignment were conducted on PCR products performed using primers for ITS regions and 26S rRNA gene beyond the D1-D2 hypervariable region.

**Conclusions**

In diagnosis of rare etiologic agents in immunocompromised hosts, mostly when no experienced medical microbiologist is promptly available, molecular tools are becoming essential for accurate species identification and proper antifungal therapy.

**FEMS-2290**

**Emerging diseases**

**PREVALENCE OF INTESTINAL PARASITIC PATHOGENS AND EVALUATION OF REAL-TIME-PCR AND ANTIGEN ELISA RESULTS FOR ENTAMOEBA HISTOLYTICA IN TURKEY, 2001-2014**

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**Background**

Intestinal parasites are two major groups including protozoa and helminths that inhabit the gastro-intestinal tract in humans. They are one of the major health problems of especially poor and under-developed countries.

**Objectives**

This study was undertaken to determine the prevalence of intestinal parasitic infection in patients with diarrhea and non-diarrhea at the Gulhane Military Medical Academy, Military Hospital in Turkey.

**Methods**

This retrospective study reviewed the hospital records of 45,638 patients admitted over a ten-year period from 2001 to 2014. During the study period, 2.33% (1859/79,537) of stool samples were tested positive for sixteen species of intestinal parasites by using standard parasitological techniques. *Giardia intestinalis* (1.47%) was the most common parasitic cause of diarrhea among the patients. Its prevalence appears to be decreasing in recent years. *Blastocystis hominis* (0.98%), *Entamoeba coli* (0.74%) ranked second and third in prevalence, respectively. *Taenia* spp (0.20%) was more common in non-diarrheal samples. The stool *E. histolytica* antigen ELISA method had 20% sensitivity and microscopy had 11.80% sensitivity compared to the results of a real-time PCR method. Majority of patients were aged between 14-50 years. Prevalence of intestinal parasitic infection was lowest in winter (11.20%), gradually increased during the spring, reached peaks of 57.34% between June and October, and gradually decreased to 2.28% in December.

**Conclusions**

These data will help provide accurate estimates of prevalence of intestinal parasites, which are crucial for the development of policies and strategies to enhance their effective control in Turkey.

**FEMS-3079**  
**Emerging diseases**

## **EMERGENCE OF CLOSTRIDIUM DIFFICILE PCR RIBOTYPE 027 IN HOSPITALS OF SILESIA, POLAND**

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### **Background**

According to 2014 ECDC surveillance an reemergence of complicated *Clostridium difficile* infection (CDI) caused by PCR 027 ribotype strains was found.

### **Objectives**

The aim of this study was to determine the incidence of CDI in 20 Silesian hospitals and to characterize *C. difficile* isolates from clinical cases.

### **Methods**

In March 2013 to May 2014, 166 stools from patients with clinically suspected CDI were tested by ESCMID algorithm. GDH was tested by CDIFF QUICK CHEK COMPLETE. GDH-positives were examined for toxins by EIA (toxin A/B) and molecular method (IllumiGene) and cultured anaerobically. Antibiotic susceptibility (MIC for 11 antibiotics) was tested with E-tests, (EUCAST interpretation).

### **Conclusions**

Of 160 GDH-positive samples, 140 *C. difficile* strains were isolated, 110 were PCR ribotyped: 91 (82.7%) belonged to PCR 027 ribotype, three (2.7%) of 176 and 014, two (1.8%) of 010 and 1 strain (0.9%) each of 001, 018, 020 and 046. Seven (6.4%) isolates were non-typeable. All 91 PCR 027 strains were susceptible to vancomycin, amoxicillin/clavulanate and piperacillin/tazobactam and resistant to moxifloxacin, ciprofloxacin, imipenem and erythromycin; 42,9% (39/91) showed resistance to clindamycin. Of 91 isolates tested, 31.9% had MIC > 2 mg/l to metronidazole (ECOFF MIC=2 mg/l) and were resistant to rifampicin.

We confirmed the importance of an appropriate algorithm for rapid CDI diagnostics. The high incidence of *C. difficile* 027 ribotype is in agreement with recent reports from other countries, though elevated MIC values to metronidazole warrants further exploration.

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**FEMS-1821**

**Emerging diseases**

## **ASSOCIATION BETWEEN PCR DETECTION RATES AND SEROLOGICAL RESULTS IN PATIENTS WITH SYPHILIS**

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### **Background**

Syphilis is a sexually transmitted disease caused by *Treponema pallidum* subsp. *pallidum*. Serological methods are considered to be the standard in syphilis diagnostics. PCR based tests for detection of treponemal DNA are being used more frequently although not routinely.

### **Objectives**

To determine whether the results of PCR detection of treponemal DNA correlate with the results of syphilis serology.

### **Methods**

Swab (n = 179) and whole blood (n = 260) samples were collected from syphilis-seropositive patients during 2004 to 2013 in the Czech Republic and underwent PCR for detection of treponemal DNA (*polA*, *tmpC*, TP0136, TP0548 and 23S rRNA genes). Several methods (RPR, VDRL, TPHA, TPPA, ELISA IgM and IgG tests and Western blot IgM and IgG tests) were used to test patients serology. Statistical analysis was conducted for all serological tests and results of PCR for both swabs and whole blood samples.

### **Conclusions**

PCR detection of treponemal DNA was more frequent in swabs (78%) compared to whole blood samples (41%). Western blot analysis revealed that patients with PCR-positive swabs ( $n = 118$ ) were more frequently IgM positive ( $p = 0.0002$ ). Analysis of 95 patients where both whole blood and swab samples were available showed that most patients with PCR-positive whole blood had also PCR-positive swabs (89%), however, only 56% of patients with PCR-positive swabs had also PCR-positive whole blood ( $p = 0.0074$ ). Success rate of detection of all 5 tested loci correlated in swabs with lower RPR titer values. In contrary, whole blood showed the opposite.

**FEMS-2686**

**Emerging diseases**

## **REAL-TIME PCR BASED DETECTION OF TROPHYRYMA WHIPPLEI CARRIERSHIP IN THE NETHERLANDS**

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### **Background**

Whipple's disease is a rare, potentially fatal multisystemic disorder resulting from infection with the rod-shaped, gram-positive bacterium *Tropheryma whipplei*. Asymptomatic carriership is a precondition of this disease that is thought to be relatively common, but reliable data on the prevalence of carriership are sparse.

### **Objectives**

To investigate the prevalence of asymptomatic *T. whipplei* carriership in the Dutch population.

### **Methods**

364 fecal samples were tested for presence of *T. whipplei* DNA using three different real-time PCR's: PCR 1, a PCR targeting a repetitive chromosomal locus, (Fenollar e.a. 2004. JCM 42:401-403), PCR 2, targeting the *rpoB*-gene (Moter e.a. 2013. JCM 51:3858-3861), and PCR 3, a newly developed PCR targeting a unique VNTR region in the genome of *T. whipplei* (this study). To solve discrepancies between PCR reactions the identity of the amplicons were determined by sequence analysis.

### **Conclusions**

The number of positive samples varied significantly between the three PCR reactions with PCR 1 finding 46/364 (12.6%), PCR 2 23/364 (6.3%), and PCR 3 21/364 (5.8%) positives. Only 19/364 (5.2%) samples were found positive in all three PCR tests. Sequencing data of the samples suggests that none of the 27 discrepant samples of PCR 1 contained sequences that were of *T. whipplei* origin. Also 3/4 discrepant samples for PCR 2 did not contain *T. whipplei* derived PCR products, while the remaining discrepant sample did. For PCR 3 sequencing of the two discrepant revealed one true positive (shared with PCR2) and one false positive. This suggests a true prevalence of 20/364 (5.5 %).



**FEMS-2116**  
**Emerging diseases**

## **OUTER MEMBRANE VESICLES AS PLATFORM VACCINE PRODUCT**

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### **Background**

Outer membrane vesicles (OMVs) are released spontaneously during growth by many Gram-negative bacteria. OMVs have pathogen-like properties inducing a broad protective immune response in hosts.

Classical vaccines were generally ill-defined products but recent recombinant subunit vaccines are developing towards well-characterized pathogen-like structures. Progress in immunology and microbiology can facilitate selection of components that should be or not be present in a platform OMV vaccine.

### **Objectives**

Evaluation of OMVs from *Neisseria meningitidis* as platform vaccine product.

Characterization of the vaccine OMV product with identified structure-function relationship.

### **Methods**

A number of different assays, such as ELISA, DLS, SDS-PAGE and Biacore aid to predict the OMV quality in the accepted potency assay, the Serum Bactericidal Assay (SBA).

For most cases, genetic engineering of the OMV-producing bacteria can be used to improve their application as vaccines. Used modifications include (I) removal of capsular structures and anchor proteins, (II) overexpression of specific antigens, (III) lipopolysaccharide (LPS) modification, (IV) retention of antigens in the membrane that can be secreted, (V) removal of unwanted immune-modulating components.

Examples of these approaches will be given.

### **Conclusions**

The increased knowledge in microbiology and immunology of OMV from bacterial pathogens will enable the development of well-characterized OMV platform vaccine products, as illustrated for *Neisseria meningitis* OMVs.

FEMS-1618

Emerging diseases

## THE INCIDENCE OF INFECTION BY BORRELIA BACTERIA AFTER A TICK BITE IN AREAS OF SWEDEN AND FINLAND

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### Background

Lyme borreliosis (LB) is a common and increasing tick-borne disease in Europe caused by the *Borrelia* bacteria. The risk of acquiring a *Borrelia* infection after a tick bite is not fully known. The transmission of *Borrelia* bacteria from ticks to humans may depend on many factors such as tick-species, life-stage, duration of tick-feeding, *Borrelia* species, number of *Borrelia* bacteria, and site for infestation.

### Objectives

We investigated the incidence of *Borrelia* infection after a bite by a *Borrelia*-positive tick and if the number of *Borrelia* bacteria and/or the duration of tick-feeding influenced the risk of contracting a *Borrelia* infection.

### Methods

During 2008-2009, ticks and blood samples were collected from 1546 tick-bitten persons from Sweden and the Åland Islands, Finland. Follow-up blood samples were taken three months after the tick bite. The duration of tick feeding was microscopically

estimated and *Borrelia* bacteria was detected and quantified in ticks by real-time PCR. Anti-*Borrelia* antibodies were detected in sera using ELISA tests and immunoblot.

### **Conclusions**

One out of four of the participants were bitten by a *Borrelia*-positive tick but only 10% of these developed signs of a *Borrelia* infection (seroconversion and/or LB diagnosis). Those who seroconverted removed “their” ticks significantly later than those who did not. The number of *Borrelia* bacteria in the ticks did not explain the risk of seroconversion. The risk of developing a *Borrelia* infection after a bite by a *Borrelia*-positive tick is small but increases with the duration of tick feeding.

**FEMS-2478**

**Environmental microbiology**

**TOXIN RESISTANCE IN AQUATIC FUNGI POSES ENVIRONMENTALLY FRIENDLY REMEDIATION POSSIBILITIES: A STUDY ON THE GROWTH RESPONSES OF *M. HIEMALIS* EH5 AGAINST MC LR, BMAA AND CYN**

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**Background**

Cyanobacteria produce various harmful secondary metabolites, which pose a serious global threat to aquatic ecosystems and human health. Biodegradation is a hot topic of water purification research and offers especially an environmentally friendly remediation strategy.

**Objectives**

The water fungus *Mucor hiemalis* EH5 (*M. hiemalis* EH5) represents a promising biodegrading candidate, as previous studies have shown its ability to breakdown the herbicide isoproturon based on natural metabolic processes and further has been characterized to be resistant to environmental perturbations, such as low temperatures and H<sub>2</sub>S/sulfide pollution, which would facilitate its application even under extreme environmental conditions.

**Methods**

Herein we elucidate the effect of three different cyanotoxins, the hepatotoxin microcystin-LR (MC-LR), the neurotoxin  $\beta$ -N-methylamino-L-alanine (BMAA) and the cytotoxin cylindrospermopsin (CYN), on the susceptibility of *M. hiemalis* EH5 using an adaptation of the Kirby-Bauer disk diffusion assay, and the influence on fungal growth and biomass production *via* radial extension and dry weight measurements.

Additionally, we established an optimized strategy for the individual cyanobacterial toxin extraction from the vegetative part of *M. hiemalis* EH5.

**Conclusions**

The fungal microorganism showed a fast adaptation behavior and strong resistance towards the toxins. No significant differences in terms of growth were perceived compared to an untreated control. This indicates that the cyanobacterial toxins have no toxic effect on the fungus and that the organism can grow and develop undisturbed in their presence. Our results suggest that *M. hiemalis* EH5 is an ideal organism to be tested as a biodegrading system for the remediation of cyanobacterial toxins from contaminated waters.

**FEMS-1290**

**Environmental microbiology**

**TREE SPECIES AFFECT FUNGAL COMMUNITY AND FUNCTIONAL DIVERSITY  
RELATED TO LIGNOCELLULOSE DEGRADATION IN FOREST SOILS**

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**Background**

Forest ecosystems play an essential ecological function by storing up to two thirds of the organic carbon. Before being stored in below-ground a large part of this carbon is contained in litter. Therefore, degradation of plant biomass is an essential process for the proper functioning of forest soils and terrestrial carbon cycling. Soil microorganisms and especially saprotrophic fungi are the principal actors of this process as they produce a wide range of extracellular enzymes involved in the decomposition of the main plant organic polymers. Nature of litter is different depending on tree species and affects diversity and composition of fungal community. We therefore hypothesized that fungal communities selected by the different tree species express specific lignocellulolytic enzymes.

**Objectives**

We simultaneously analyzed the effect of tree species (beech vs spruce) on the taxonomic composition of the active fungal community in soils using the Elongation factor I (EF1alpha) and on the diversity of expressed fungal genes encoding three main families of Carbohydrate-Active Enzymes (CAZymes) involved in the degradation of the lignocellulose (GH7, GH11 and AA2).

**Methods**

Environmental cDNA, generated from soil extracted RNA, were used as template to amplify the targeted genes using family-specific primers. All amplicons were sequenced by high-throughput sequencing using Illumina Miseq technology.

**Conclusions**

Results obtained revealed that only a few numbers (less than 20 percent) of taxonomic and functional clusters were detected in both forest soils. Statistical analysis (AMOVA) showed a significant tree species effect on the diversity of the transcript analyzed.



**FEMS-1883**

**Environmental microbiology**

**DIURNAL VARIATION OF BACTERIAL COMMUNITIES IN DRINKING WATER SYSTEMS OVER SMALL SCALES.**

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**Background**

The application of next-generation sequencing techniques to the study of microbial communities that inhabit Drinking Water Systems (DWSs) has revealed the presence of a diverse and abundant microbiome. To our knowledge, the seasonal, weekly and monthly variations of the DW microbiome have been explored, while information on diurnal variation is still lacking. Similarly, sampling efforts mostly focus on covering large spatial scales in the DW, while information on DW microbiome over small spatial scales is still limited.

**Objectives**

To investigate the variabilities in the drinking water microbiome originating from: (a) diurnal water use patterns, and (b) small-scale spatial effects.

**Methods**

Triplicate samples of disinfected bulk water (15 to 18L) were taken from 5 sampling locations in Glasgow over 24 hours, in six time periods to capture diurnal effects. The samples were filtered through sterile 0.22µm pore size filters and subject to DNA extraction, triplicate 16S rRNA targeted PCR amplification followed by Illumina MiSeq sequencing. Sequence processing was conducted in Mothur using a previously reported protocol. Statistical analyses were conducted using Mothur and R.

**Conclusions**

Significant differences in richness over a 24-hour period were observed in 3 of the 5 sampling locations. Across sampling locations, a significant difference for the time periods corresponding to higher demand and flows in the system was observed, while differences were not significant for time periods corresponding to lower demand and flows in the system. For all the sampling locations, a change in the community structure and membership throughout the day was observed.



**FEMS-0525**

**Environmental microbiology**

**SUBLETHAL 2,4-DICHLOROPHENOXYACETIC ACID REMODELS CELL ENVELOPE AND ALTERS VITAL PATHWAYS OF CELL METABOLISM IN *E. COLI* AND *RHIZOBIUM LEGUMINOSARUM***

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**Background**

There is a growing need to characterize the effects of environmental stressors at the molecular level on model organisms with the ever increasing number and variety of anthropogenic chemical pollutants. The herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), one of the most widely applied pesticides in the world, is known to have undesirable effects on nontarget species, but the specific molecular details of these effects at sublethal levels are unknown.

**Objectives**

The model bacterium *Escherichia coli* and the nitrogen fixing, beneficial soil organism *Rhizobium leguminosarum* viciae 3841 (*Rlv*) were used to characterize the specific stress response mechanisms during sublethal 2,4-D exposure.

**Methods**

We designed a unique platform combining advanced microscopy with metabolomics to determine the sublethal effects of 2,4-D on cell morphology, surface molecular organization and physical properties, and specific target pathways in the metabolic network.

**Conclusions**

*E. coli* BL21 and a selection of genotypically diverse *E. coli* strains isolated from the environment exhibited filamentous phenotype and envelope remodelling during low level 2,4-D exposure. Sublethal 2,4-D altered vital pathways of cellular metabolism most of which could be attributed to oxidative stress, consistent with increased reactive oxygen species (ROS). *Rlv* exposed to 2,4-D also showed accumulation of ROS and carbonylated proteins coinciding with adaptive changes to integral pathways of cellular metabolism, envelope remodelling and the potential to assimilate 2,4-D. This study identifies biomarkers of 2,4-D exposure in *E. coli* and *Rlv* and offers valuable insights into the mode-of-action of 2,4-D at sublethal levels in soil bacteria.

**FEMS-2016**

**Environmental microbiology**

**EFFECT OF OCEAN ACIDIFICATION ON RHODOLITH HOLOBIONTS : FREE-LIVING CORALLINE ALGAE AND THEIR ASSOCIATED MICROBIOTA**

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**Background**

Rhodoliths are free-living coralline algae (*Rhodophyta*, *Corallinales*) that are ecologically important for the functioning of marine environments. They form extensive beds distributed worldwide, providing a habitat and nursery for benthic organisms and space for fisheries, and are an important source of calcium carbonate. Rhodolith beds are large carbon sinks, but the growth of the Rhodolith holobiont may be affected by lower pH, which is predicted to occur in the near future. The term holobiont refers to any organism and all of its associated symbiotic microbes (parasites, mutualists, synergists and amensals), including endobionts and epibionts that perform diverse ecological roles. A holobiont occupies and adapts to an ecological niche, and is able to employ strategies unavailable in any one species alone when challenged by environmental perturbations.

**Objectives**

The main goal of this research was to perform an experiment to test the effects of higher pCO<sub>2</sub> (lower pH) on Rhodoliths holobionts in order to analyze whether the microbial community associated with Rhodoliths is perturbed with a change in pH.

**Methods**

The effect of climate changes, indeed increasing acidification of oceans, on Rhodolith holobiont growth may be due to dissolution of their calcium carbonated skeleton or a change in the microbial community and this was investigate through physiological assays (photosynthesis and calcification) and metagenomics (taxonomical and functional diversity of the holobiont).

**Conclusions**

The outcomes of the research are an increased understanding of microbes associated with Rhodoliths and new hints on how the holobiont might respond to global climate changes.

**FEMS-2773**

**Environmental microbiology**

## **METABOLOMIC PROFILES OF MARINOBACTER ISOLATE J5B1 GROWN ON DIFFERENT NAVAL FUELS**

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### **Background**

Replacement of crude oil with its synthetic and biological equivalents is important for environmental and economical reasons. It is essential to assess synthetically produced alternative fuels in terms of bio/degradability and storage conditions in comparison to crude oils.

### **Objectives**

This study investigates the growth of a model marine microorganism in seawater in the presence of different naval fuels and its impact on corrosion of 1018 carbon steel storage tanks.

### **Methods**

Cultures of *Marinobacter* isolate J5B1 were incubated in sterilized Key West seawater augmented with different naval fuels, i.e., F76, JP5, FT-F76 and JP5-Camelina. All cultures contained 1018 carbon steel coupons. Bulk phases of each incubation were sampled periodically for cell count and metabolomes over a period of 80 days. Scanning electron microscopy was employed to characterize surfaces of exposed coupons. Polar organic compounds in neat fuels and their water extracts were analyzed using HPLC-Mass Spectrometry.

### **Conclusions**

*Marinobacter* isolate J5B1 grew in the presence of all tested fuels. HPLC-MS analysis revealed that all neat fuels shared 84 compounds while 21 compounds were common in water extracts of fuels. Fuel F76 was the most and JP5-Camelina was the least diverse in terms of the abundance of polar compounds. Seawater alone could maintain the growth of *Marinobacter* and water soluble compounds of JP5-Camelina were readily utilized by this isolate. Quaternary ammonium compounds detected in F76 fuel were most likely the reason for stunted bacterial growth. While general corrosion of steel coupons was noted in all incubations the highest level of localized attack was observed in cultures augmented with F76.



**FEMS-2520**

**Environmental microbiology**

**GENOMIC, PROTEOMIC AND MORPHOLOGICAL CHARACTERIZATION OF TWO NOVEL BROAD HOST LYTIC BACTERIOPHAGES (PHI)PD10.3 AND (PHI)PD23.1 INFECTING SOFT ROT ENTEROBACTERIACEAE**

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**Background**

*Pectobacterium* spp. and *Dickeya* spp. are necrotrophic bacterial pathogens of many important crops worldwide. The effective strategies to control pectinolytic bacteria have not yet been developed. Consequently, the management of *Pectobacterium* spp. and *Dickeya* spp. is based mainly on the exclusion of infected plant material and the use of hygienic practices during potato cultivation and storage.

**Objectives**

This study reports on the isolation and characterization of broad host lytic bacteriophages able to infect the dominant *Pectobacterium* spp. and *Dickeya* spp. affecting potato in Europe viz. *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc), *P. wasabiae* (Pwa) and *Dickeya solani* (Dso) with the objective to assess their potential as biological disease control agents.

**Methods**

The lytic bacteriophages were isolated from potato samples collected in different potato fields in Poland. Transmission electron microscopy was used to study bacteriophage morphology. The phages were characterized for optimal multiplicity of infection, the rate of adsorption to the bacterial cells, the latent period and the burst size. They were also genotypically characterized with RAPD-PCR and RFLP techniques. The structural proteomes of both phages were obtained by fractionation of phage proteins by SDS-PAGE. Phage protein identification was performed by liquid chromatography-mass spectrometry (LC-MS) analysis. Pulsed-field gel electrophoresis (PFGE), genome sequencing and comparative genome analysis were used to gain knowledge of the length, organization and function of the (phi)PD10.3 and (phi)PD23.1 genomes.

**Conclusions**

This is the first study dealing with isolation and characterization of broad host lytic bacteriophages able to infect dominant *Pectobacterium* and *Dickeya* spp. in potato in Europe.



**FEMS-0601**

**Environmental microbiology**

**HIGH ABUNDANCE OF LEADERLESS MESSENGER RNA TRANSCRIPTS AND A NOVEL RADIATION RESPONSE MECHANISM IN RADIORESISTANT BACTERIUM *DEINOCOCCUS DESERTI***

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**Background**

*Deinococcus* bacteria tolerate very high doses of gamma and UV radiation and other massive DNA damage- and oxidative stress-generating conditions.

**Objectives**

To better understand the mechanisms underlying extreme radioresistance, we characterized the transcriptome of *Deinococcus deserti*. Furthermore, we investigated how IrrE, a protein essential for radioresistance, is involved in upregulation of various genes following irradiation.

**Methods**

Differential RNA sequencing was performed, allowing genome-wide identification of transcription start sites (TSSs). The function of IrrE, which contains a conserved domain of unknown function (COG2856/DUF955), was analysed using several genetic and biochemical experiments.

**Conclusions**

Strikingly, 60% of the identified mRNAs in *D. deserti* is leaderless, with the TSS exactly at or very close to the translation initiation codon (1). Such proportion of leaderless mRNAs is unprecedented for a bacterial species. Numerous additional leaderless mRNAs predicted to encode small peptides were identified, providing a new explanation for the accumulation in the cell of small peptides important for protection of proteins against oxidation and thus for radioresistance. Several novel highly radiation-induced genes were also found. The mechanism by which IrrE is involved in upregulation of DNA repair and other genes has remained unknown for many years. We have now demonstrated that IrrE is a metalloprotease that cleaves and inactivates repressor protein DdrO (2). In *D. deserti*, this cleavage is stimulated by irradiation.

(1) *Genome Biol Evol* (2014) 6: 932-948.

(2) *Mol Microbiol* (2014) 94: 434-449.





**FEMS-1243**

**Environmental microbiology**

**NEXT GENERATION SEQUENCING FOR THE IDENTIFICATION OF  
FILAMENTOUS BACTERIA IN INDUSTRIAL ACTIVATED SLUDGE PLANTS**

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**Background**

Filamentous bacteria are mostly identified in activated sludge. Under specific conditions these organisms can outcompete other bacteria inducing process disturbances underlining the importance of a better comprehension and understanding on the growth of these bacteria to develop effective control measures.

**Objectives**

Identification of filamentous bacteria present in industrial WWTPs and evaluation of influencing factors on the growth of these bacteria.

**Methods**

Activated sludge samples from two industrial WWTPs in Germany were collected.

DNA-sequencing based PCR-product from all bacteria primer (104F, 515R) was conducted via Illumina platform.

Sequencing data was generated using a developed bioinformatics pipeline and it was further analyzed by BLAST.

Biodiversity analyses using MEGA 5.1 and R studio 3.1.0 were performed.

**Conclusions**

In total 432 different gene sequences were identified showing 17 sequences related to bulking and foaming bacteria as described in Guo and Zhang (2012).

The abundance of filamentous bacteria was calculated as the percentage of total reads. *Chryseobacterium* sp. *Iso-52* (24.82 %), *Candidatus Microthrix parvicella* (*M. parvicella*) clone OTU-5-40m.ABB (21.77 %), *Chloroflexi bacterium* ET1 (15.61 %) and *Gordonia* sp. YIM 100324 (11.67 %) were the most dominant filamentous organisms found.

Phylogenetic analysis revealed a close relationship of *M. parvicella* and *Gordonia*.

Canonical correspondence analysis (CCA) indicated positive correlations to sludge age and wastewater influent for *M. parvicella* and *Gordonia*, while dissolved oxygen (DO), effluent phosphorous and ammonia concentration, nitrate sludge loading showed a negative impact. It was also observed that the occurrence of *M. parvicella* and *Gordonia* is connected to high sludge volume index (SVI) and floating sludge

fraction (AVA) values.

**FEMS-0991**

**Environmental microbiology**

## **SURVIVAL OF VIABLE LISTERIA MONOCYTOGENES CELLS DURING LIVESTOCK MANURE STORAGE DETERMINED BY MOLECULAR AND CULTURE ASSAYS**

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### **Background**

Pig manure is usually stored in pits before spreading on agricultural land. It may also be biologically treated, leading to a liquid fraction stored in a lagoon (lagoon effluent). The impact of the storage on the persistence of pathogens depends on environmental factors and on intrinsic factors of the pathogens including their ability to enter into a viable but non cultivable (VBNC) state.

### **Objectives**

This study aims to compare the behaviour of *Listeria monocytogenes* which can enter VBNC state, in pig manure and in lagoon microcosms.

### **Methods**

Two strains of *L. monocytogenes* belonging to different serogroups were isolated from two farms. They were inoculated in flasks containing two manures and two lagoon effluents stored at 8 and 20°C during 63 days. The bacteria were quantified by cultural method, qPCR and qPCR associated with Propidium MonoAzide which quantifies both cultural and VBNC cells.

### **Conclusions**

Regardless their origin and their serogroup, both strains showed similar kinetics of decrease. The lower temperature increased the persistence of the strains. An effect of the matrix was observed not only between manures and lagoon effluents but also within a same type of matrix with similar chemical composition, suggesting that biotic factors may play a role in the persistence of *L. monocytogenes*. The VBNC appeared within the first hours of contact regardless the matrix. Nevertheless, their proportion increased over time in the effluent lagoon.

This study highlights that lagoon effluents used for watering plants, creates more favorable conditions than manure for VBNC cells of *L. monocytogenes*.



**FEMS-1676**

**Environmental microbiology**

## **DENITRIFICATION AND NITROUS OXIDE PRODUCTION IN SOILS IN THE HAMPSHIRE RIVER AVON CATCHMENT IN THE UNITED KINGDOM**

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### **Background**

Microbial denitrification can lead to a loss of N from soils to atmosphere as N<sub>2</sub> or N<sub>2</sub>O. However, the magnitude of the soil N sink and environmental factors regulating denitrification are essentially unknown in UK catchments.

### **Objectives**

This study aims to examine seasonal and spatial variation of denitrification and N<sub>2</sub>O production and environmental factors regulating the processes and constrained the magnitude of N loss by denitrification.

### **Methods**

Rates of denitrification and N<sub>2</sub>O production were measured in the laboratory by <sup>15</sup>N isotope enrichment technique in grassland soils in three sub-catchments with contrasting geologies (clay, greensand and chalk) in the River Avon catchment at four seasons from August 2013 to May 2014,

### **Conclusions**

Denitrification and N<sub>2</sub>O production varied seasonally and spatially with annual mean rates of denitrification of 0.025, 0.265, and 0.210 µg N.g<sup>-1</sup> dry soil.day<sup>-1</sup> respectively, and of N<sub>2</sub>O production of 0.870, 0.547 and 0.620 ng N.g<sup>-1</sup> dry soil.day<sup>-1</sup> respectively at clay, Greensand and chalk sites. Denitrification rates were higher in August and November and higher rates of N<sub>2</sub>O production were observed in August and May.

Rates of denitrification and N<sub>2</sub>O production, measured at four temperatures (4, 10, 20 and 30°C) and three levels of soil water concentration (57.5%, 77.5% and water saturation), increased with temperature and soil water content.

The dominating environmental factors for denitrification and N<sub>2</sub>O production were soil pH, soil water content and temperature. It is estimated that annual losses of N by

denitrification were 11.8, 125.8 and 99.7 kg N per hectare at clay, greensand and chalk sites respectively.

**FEMS-2738**

**Environmental microbiology**

**EFFECT OF ENERGY CROP CULTIVATION AND FERTILIZATION ON SOIL  
ARCHAEAL COMMUNITY AND ITS POTENTIAL OF METHANOGENESIS IN  
ABANDONED PEAT EXTRACTION AREA**

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**Background**

Peat is an important resource and is being extracted widely from regions rich in peatlands, which inevitably leads to the problem of abandoned peatlands. These areas emit greenhouse gases (CH<sub>4</sub>, N<sub>2</sub>O, CO<sub>2</sub>) for decades if restoration action is not implemented. The potential of methane production of abandoned peatlands can be evaluated by the abundance of *mcrA* genes which encodes the alpha-subunit of the enzyme that catalyses the last step in CH<sub>4</sub> synthesis in all methanogens.

**Objectives**

The purpose of this study is to assess the effect of energy crop (*Phalaris arundinacea* L.) cultivation and fertilisation on the abundance of soil bacterial and archaeal 16S rRNA genes, and to evaluate the methanogenic potential of the microbial communities in the soils of the experimental plots on the abandoned peat extraction area.

**Methods**

The experiment was carried out in 2012-2014 and samples were collected from 12 experimental plots at the vegetation period. Besides the widely used statistical methods, linear mixed effects modelling was used to test relationships between gene parameter values and chemical variables and in cases of different grouping factors.

**Conclusions**

Various physicochemical parameters changed the abundance of peat methanogenic community in vertical profiles. Methane emission rates correlated with the abundance of peat deeper layers methanogenic community in uncultivated plots while without *Phalaris* cultivation there are less constraining factors. These findings are important in the assessment of global warming potential while using abandoned peat extraction areas to produce biomass for energy.





**FEMS-0658**

**Environmental microbiology**

## **BIODECOLORIZATION OF INDUSTRIAL TEXTILE DYES BY A NEWLY ISOLATED BACILLUS SP.JG12 FROM TEXTILE EFFLUENTS**

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### **Background**

Release of effluents from textile dyeing and printing industries prior to proper treatment is a vital source polluting water and soil. A number of structurally dissimilar industrial azo dyes are present in textile effluents.

### **Objectives**

To isolate, screen and characterize a bacterial isolate having potential to decolorize and degrade industrial textile dyes from textile effluent. To investigate the effects of various environmental and nutritional parameters on decolorization activity of that isolate on a specific dye under lab condition. To study phytotoxic effects caused by decolorized and degraded dye products.

### **Methods**

Decolorization and degradation study of selected dye was performed using UV Visible spectroscopy and HPLC analysis. Germination assay of seeds of some selected crops was performed to investigate the toxic effects of decolorized and degraded dye products,

### **Conclusions**

We were able to screen a bacterial isolate with decolorization and degradation potential from the textile effluents. The complete decolorization of dye (150 mgL<sup>-1</sup>) was achieved within 24 hrs of incubation under static culture condition (at pH7, 37°C). The maximum decolorization was found in presence of Yeast extract as a source of carbon (at pH7±0.2, 37°C) Static culture condition. Seeds irrigated with decolorized dye sample (under static culture condition) show inhibition in germination. Inhibition of seed germination was not observed in case of seeds irrigated using dye treated under sequentially static-shaking culture conditions did not show.

**FEMS-1547**

**Environmental microbiology**

## **GENE CAPTURE BY HYBRIDIZATION AS A STRATEGY FOR TARGETED MICROBIAL GENOME RECONSTRUCTION**

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### **Background**

Microbial communities show the greatest organisms diversity on earth. Culture-independent molecular approaches targeting ssu ribosomal RNA genes have revealed this extraordinary diversity. However, amplicons cannot establish the link between microbial communities structure and realized metabolic functions, limiting the comprehension of micro-organisms roles. Despite the advent of the current ultra-high throughput sequencing, efficient assembly of sequencing data from metagenomic samples remains difficult and complete genome reconstruction has been principally realized for dominant micro-organisms. Single cell sequencing strategies have also been developed to overcome these limitations and to access less abundant micro-organisms but are not always easily practicable.

### **Objectives**

Based on a solution hybrid selection method combined with next generation sequencing, we developed an innovative gene capture approach to enable the reconstruction of large genomic regions or even complete microbial genomes from complex environments.

### **Methods**

A first gene capture using highly specific short probes targeting 16S rRNA gene was applied on metagenomic DNA to obtain large specific probes (several kbp) spanning the biomarker and the unknown flanking regions of the microbial species of interest, *Roseobacter denitrificans*. Generated long probes were then used to capture very large DNA fragments of *R. denitrificans* within a mix of bacterial gDNA. Real-time PCR performed on captured DNA revealed a significant enrichment in targeted 16S rRNA gene, surrounding genes and even several Mbp away genes, suggesting the capture of *R. denitrificans* complete chromosome.

### **Conclusions**

Sequencing of captured DNA allows identification of unknown sequences and reveals new genetic associations. This promising approach will facilitate linking identity and associated functions.



**FEMS-1481**

**Environmental microbiology**

**THE IMPACT OF SEPTIC SYSTEMS AND WATERSHED LEVEL CHARACTERISTICS ON STREAM FAECAL POLLUTION IN SUBURBAN WATERSHEDS IN GEORGIA, USA**

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**Background**

A quarter of housing units in the USA use septic systems for wastewater treatment and disposal. As widely as septic systems are used, their watershed level impact on water quality is poorly understood.

**Objectives**

The objective of this study was to determine the impact of septic systems on water quality and in so doing identify watershed level characteristics that influence their impact.

**Methods**

Water samples, collected from 24 well characterized watersheds with varying septic density, were analyzed for fecal indicator bacteria to quantify pollution levels and identify seasonal trends. The watersheds represent a gradient of land-use conditions from low to high density of septic systems, as well as developed to undeveloped uses. Pollutant sources were then tracked using *Bacteroides* sp. genetic markers.

**Conclusions**

Our findings indicated statistically significant interaction between septic density and season for enterococci count ( $P = 0.005$ ) and stream yield ( $P = 0.04$ ). Seasonal variations in bacterial count and stream yield were also observed, with significant differences between spring–winter and summer–winter. Multiple linear regression models suggested that 4 watershed characteristics (septic density, median distance of septic systems to stream, % developed area and forest cover) and water temperature could explain about half of the variability in bacterial count and yield in spring and summer. There was a significantly positive correlation ( $R = 0.67$ ) between septic density above 77 units/km<sup>2</sup> and human-specific marker yield (indicator of septic contribution). Overall study conclusion was that septic density strongly influences fecal pollution but its impact is dependent on season and watershed characteristics.

**FEMS-1508**

**Environmental microbiology**

**ANAEROBIC DEHALOGENATING BACTERIA RELATED TO DESULFOLUNA SPONGIIPHILA FORM A COSMOPOLITAN GROUP WIDELY DISTRIBUTED IN ORGANOHALIDE-CONTAINING SPONGES**

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**Background**

The marine environment is a rich source of biogenic organohalides produced by a diversity of marine organisms, such as sponges that produce a vast array of halogenated bioactive compounds as secondary metabolites. These organohalogen compounds in turn appear to select for bacteria that can utilize them as a source of energy.

**Objectives**

Our objectives are to investigate the dehalogenating bacterial populations within the sponge animal with the overall goal of understanding the roles and metabolic activities of this endomesohyl microbiota.

**Methods**

We enriched for bromophenol dehalogenating bacterial symbionts from a range of sponge species collected at different sites in the Mediterranean, Atlantic and Pacific, and applied an integrated cultivation and molecular analysis-based approach to analyze the bacterial communities.

**Conclusions**

We demonstrated the widespread distribution of anaerobic reductive debrominating bacteria in different marine sponges. We isolated a novel bacterial species, *Desulfoluna spongiiphila*, from Mediterranean sponges that grows by respiratory reductive dehalogenation. Using a cultivation and molecular analysis-based approach we demonstrated that *D. spongiiphila* and its close relatives form a cosmopolitan group widely distributed in organohalide-containing sponges across different geographic locations. The sponge-associated dehalogenating bacteria can operate *in vivo* and impact the fate of brominated organics. This new bacterial species group is an excellent model system to study a 'chemically-driven' endosymbiotic relationship and one possible origin of organohalogen respiration. Organobromine-rich sponges

appear to provide a specialized, possibly ancient, habitat for organohalide-respiring microbes, which mediate a cycling of organohalide compounds within the sponge animal.

**FEMS-0616**

**Environmental microbiology**

## **METHANESULFONATE (MSA) CATABOLIC GENES FROM MARINE AND ESTUARINE BACTERIA**

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### **Background**

Quantitatively methanesulfonate (MSA) is very relevant in the global biogeochemical sulfur cycle. MSA utilization by bacteria as a source of carbon and energy has been described and a specific enzyme, methanesulfonate monooxygenase (MSAMO), has been found to perform the first catabolic step of its oxidation. Other proteins, seemingly involved in the import of MSA into bacterial cells, have been reported.

### **Objectives**

In this work we looked for the genes encoding the large subunit of the MSAMO (*msmA*) and the periplasmic component of the import system (*msmE*) in the genomes of MSA-enriched bacteria and also in metagenomic DNA from seawater, in order to extend our knowledge on these genes as ecofunctional genetic markers of MSA degradation.

### **Methods**

PCR approaches were implemented and the resulting products were cloned and sequenced. Two novel marine MSA-isolates were also submitted to whole genome sequencing.

### **Conclusions**

In conclusion, novel *msmA* and *msmE* sequences were obtained, and two full *msm* operons were found in the genomes of the novel marine strains. Clearly, the *msmA* genes (and derived proteins) show less sequence variability than *msmE*. This, added to the apparent conservation of a peculiarly long Rieske-associated motif, reinforces the value of *msmA* as ecofunctional indicator for methanesulfonate cycling by bacterial natural communities. In the line of previous observations, our results also show that high-GC strains are somehow favoured during enrichment and isolation of MSA-utilizing bacteria, illustrating the misrepresentation of natural populations that

culturing may entail.



**FEMS-0482**

**Environmental microbiology**

**BIOREMEDIATION: A TOOL OF MULTIPLE HEAVY METALS RESISTANT  
ENDOPHYTIC FUNGI FOR PHYTOSTIMULATION**

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**Background**

Heavy metal pollution is of great concern in countries like Pakistan where a huge proportion of human population is exposed to it. Bioremediation is one of the most viable and efficient solution for the problem.

**Objectives**

Purpose of the current study was to isolate endophytic fungi from corn plants and screen them for phytostimulation under heavy metal stress.

**Methods**

Endophytic strain (MHR-23) was identified as *Paecilomyces formosus* by 28 S rDNA and internal transcribed spacer (ITS) sequence homology. The strain effectively tolerated up to 2000 µg mL<sup>-1</sup> of these heavy metals. Adverse effects of metals were obvious in the form of gradual decrease of fungal growth by increasing the concentration of heavy metals. The strain was able to remove 60-87 % of metals (300 µg mL<sup>-1</sup>) from PDA broth. The strain removed metals by biotransformation and/or accumulation of heavy metal in its hyphae. Growth of corn stressed with heavy metals was restored by the application of MHR-23. Accumulation of heavy metals by corn stem and leaves was significantly reduced ( $p < 0.05$ ) by the application of MHR-23.

**Conclusions**

It may be concluded that MHR-23 is an excellent candidate to be used as biofertilizer in fields affected with heavy metals.

**FEMS-2724**

**Environmental microbiology**

**GENOME DATA MINING IN DETERMINATION OF GENES INVOLVED IN THE ANTIMICROBIAL ACTIVITY OF PSEUDOMONAS SP. P482 AGAINST PLANT PATHOGENIC BACTERIA AND FUNGI**

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**Background**

To survive in a competitive environment, the plant-associated *Pseudomonads* produce a set of biologically active compounds, many of them known for their antimicrobial properties. *Pseudomonas* sp. P482, a tomato rhizosphere isolate, shows antagonistic activity towards a spectrum of plant pathogenic bacteria and fungi.

**Objectives**

In this study, we aimed to apply a genome data mining approach to determine the genetic background of the antibacterial activity of the P482 strain.

**Methods**

The genome sequencing of P482 was performed. Bioinformatics tools such as the antiSMASH and BAGEL3 were employed to analyze the resulting data and to determine the 'candidate' genes potentially involved in the antimicrobial activity of the P482. The 'candidate' genes were subjected to site directed mutagenesis with the use of a suicide vector. The obtained mutants were tested for the lack of antimicrobial activity towards selected strains of plant pathogenic bacteria and fungi.

**Conclusions**

A draft genome sequence of P482 was obtained and automatically annotated. The BAGEL3 analysis yielded no putative bacteriocins. The antiSMASH analysis revealed 5 biosynthetic clusters encoding biosynthetic genes potentially involved in the synthesis of antimicrobial factors. Knock out of the 5 major biosynthetic genes, one per each predicted cluster, has shown that these genes are not essential for the antimicrobial activity of P482 against the soft rot bacteria. Thus, genome mining is a powerful approach yet when novel mechanism of antagonism are at stake, as in seems to be in case of the P482, it should be used in combination with alternative methods.



**FEMS-1936**

**Environmental microbiology**

**ELUCIDATING THE GENETIC BASIS FOR SULFONAMIDE DEGRADATION IN ISOLATES WITH DIVERSE PHYLOGENETIC BACKGROUND**

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**Background**

Sulfonamides antibiotics are among the most frequently prescribed antibiotics in human and veterinary medicine, and, at the same time quite recalcitrant to degradation. Therefore, sulfonamides can be commonly detected in wastewater worldwide in trace amounts. Up to now, few sulfonamide degrading bacteria stemming from different phyla have been isolated. While the degradation mechanisms in most strains have not been conclusively elucidated yet, most cases were shown to lead to analogous metabolites

**Objectives**

The intention of this study is to understand how sulfonamide degradation genes are propagated in the environment and whether the isolated sulfonamide strains have in common a similar apparatus of genes encoding degradative enzymes.

**Methods**

A set of draft genomes of sulfonamide degrading bacteria was obtained by next generation sequencing of genomic DNA of these strains and from public available data sets. The nucleotide sequence of a gene cluster related to sulfonamide degradation in *Microbacterium* sp. BR1 was compared to similar regions found in other strains.

**Conclusions**

It was found that some sulfonamide degraders contained in their genome DNA regions highly similar to that found to be responsible for degradation in *Microbacterium* sp. strain BR1, while for others, similarities to this region were too low to safely attribute the same function to the encoded enzymes. It remains to be solved

whether these distantly related genes confer degrading activity in these strains or whether completely different enzymes are involved.

**FEMS-1487**

**Environmental microbiology**

## **ROLE OF VIBRIO CHOLERAE ADHESINS IN SURVIVAL OF PREDATION BY HETEROTROPHIC PROTISTS**

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### **Background**

*Vibrio cholerae* is an opportunistic pathogen and a natural inhabitant of aquatic ecosystems. Predation by heterotrophic protozoa has a major impact on the environmental survival of *V. cholerae*. Adhesins are important virulence factors required for *V. cholerae* pathogenesis but may have evolved during long-term interactions with protozoa.

### **Objectives**

Here, we investigated the role of *V. cholerae* adhesions (TcpA, MshA, GbpA, LapA and VC1804) in resistance to predation by the surface-feeding *Acanthamoeba castellanii*.

### **Methods**

*V. cholerae* wild type stains and adhesin mutants were exposed to predation by *A. castellanii*. The grazing resistance was determined by *V. cholerae* selective grazing assays, and quantification of *V. cholerae* cells attached to the surface and/or internalized by *A. castellanii* as assessed by CFUs and microscopy.

### **Conclusions**

Data show that only VC1804 made a significant contribution to grazing resistance as the mutant was grazed more than the parent wild type. Compared to wild type strains, the MshA and VC1804 mutants exhibited significantly lower colonization efficiency for the *A. castellanii* surface, which also resulted in less cells surviving intracellularly in the amoebae. These findings further elucidate the role of *V. cholerae* adhesins in persistence in the environment and support the hypothesis that some virulence factors provide for increased fitness in the environment.

**FEMS-2447**

**Environmental microbiology**

## **THE VALUE OF *P. AERUGINOSA* IN HEALTH AND MICROBIOLOGICAL QUALITY CONTROL OF RECREATIONAL WATERS USING IN PUBLIC SWIMMING POOLS**

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### **Background**

*Pseudomonas aeruginosa* is the most significant bacteria capable of multiplying in water especially in recreational waters. Nowadays most public swimming pools use recreational water.

### **Objectives**

To assess if these swimming pools are health risk to users, eleven of public swimming pools in East and North-East of Tehran were monitored. Useful information such as flow rate of filtration of water in the pools, clarity, temperature and pH of the water were recorded at the same time of sampling too.

### **Methods**

According to the Standard Method 21th edition, Bacteriological tests such as isolation, identification and colony counts of *Escherichia coli*, coliforms, and *Pseudomonas aeruginosa* were performed on the samples. *P. aeruginosa* was isolated from 9(81.8%) of the pools. Also *P. aeruginosa* was the only predominant organism isolated from 7(63.6%) of the pools, while in the remained 2(18.2%) other, in addition to *P. aeruginosa*, high rates of total bacterial count, total coliforms and faecal coliform counts were also found.

### **Conclusions**

Results of the contaminated swimming pools revealed that chlorination was often inadequate to disinfect mentioned bacteria especially *P. aeruginosa* when high population of people had to over use of the pools. Thus results of this study, showed the importance and the value of assaying presence of indicator of *P. aeruginosa* routinely in quality control of recreational waters using in public swimming pools.

**FEMS-1798**

**Environmental microbiology**

**THE EFFECT OF NUTRIENTS ON CARBON AND NITROGEN FIXATION BY THE UCYN-A-HAPTOPHYTE SYMBIOSIS**

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**Background**

Symbioses between phytoplankton and N<sub>2</sub>-fixing microorganisms play a pivotal role in the ocean's carbon and nitrogen cycle. The abundant and widespread unicellular cyanobacteria group A (UCYN-A) was recently found to live symbiotically with a haptophyte.

**Objectives**

Here, we investigated the effect of nitrogen (N), phosphorus (P), iron (Fe) and Saharan dust additions on nitrogen (N<sub>2</sub>) fixation and primary production by the UCYN-A-haptophyte association in the subtropical eastern North Atlantic Ocean.

**Methods**

In order to determine single-cell rates of N<sub>2</sub> fixation and primary productivity, we used stable isotope incubations combined with nanoSIMS measurements. *nifH* gene expression analysis was carried out to determine the molecular regulation of N<sub>2</sub> fixation under the different nutrient conditions.

**Conclusions**

N<sub>2</sub> fixation by UCYN-A was stimulated by the addition of Fe and Saharan dust whereas CO<sub>2</sub> fixation by the haptophyte was stimulated by the addition of ammonium nitrate as well as Fe and Saharan dust. However, there were no detectable differences in *nifH* gene expression in any of the incubations. Independent of the nutrient treatment, we observed a mutual exchange of carbon and nitrogen between the UCYN-A and the haptophyte suggesting a tight coupling. However, it appears that the transfer of carbon from the haptophyte to UCYN-A requires a transfer of nitrogen from UCYN-A indicating an obligate symbiosis of this globally important diazotrophic association.



**FEMS-1433**

**Environmental microbiology**

**RESPONSE OF GEOLOGICAL RESIDENT MICROBIAL COMMUNITIES TO ADDITIONS OF NITRATES AND/OR ACETATE, MIMICKING LEACHATES OF BITUMINIZED INTERMEDIATE-LEVEL RADIOACTIVE WASTE**

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**Background**

Clay deposits such as the Opalinus Clay formation are studied as host rocks for geological disposal of high- and intermediate-level long-lived radioactive waste in several European countries. Bituminized intermediate-level long-lived radioactive waste contains, besides bitumen and radionuclides, also large amounts of organics, nitrates and sulphates. Over time, these salts will dissolve, leach and diffuse into the surrounding clay host rock, together with water soluble organic substances. To mimic the potential effect of such inorganic– and organic leachates, pulses of nitrate and/or acetate have been injected in intervals of an *in situ* experiment, called Bitumen-Nitrate-Clay interaction (BN) experiment, running at the Mont Terri geological laboratory (St. Ursanne, Switzerland).

**Objectives**

To elucidate whether the microbial communities, present in the BN experiment, are affected or involved in the observed biogeochemical changes.

**Methods**

Beside classical microbial analyses, at pivotal moments (*i.e.* before, during and after these pulse injection tests), priority was given to DNA-based molecular biology analysis methods, as these methods provide very accurate information on the composition, the metabolic capacity and possible evolution of bacterial communities in response to the nitrate and/or acetate injections.

**Conclusions**

The changes observed in the bacterial populations appeared to correlate well with the imposed physico-chemical changes. As soon as nitrate was added an overwhelming community shift appeared to nitrate reducing bacteria. If in parallel acetate was offered, the community composition did not alter that much, but the speed of nitrate reduction was increased twentyfold. This high nitrate removal speed was maintained as long as the easily consumable carbon source was abundant.



**FEMS-2046**

**Environmental microbiology**

**FUNCTIONAL CHARACTERIZATION OF METAGENOMES FROM MICROBIAL COMMUNITIES IN PRISTINE AND CHRONICALLY-POLLUTED HARBOR WATERS IN RELATION TO HYDROCARBON POLLUTION**

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**Background**

The phylogenetic composition of bacterial communities in chronically-polluted harbor waters in Mallorca Island (Spain) has been shown to differ from those in nearby clean waters, with a characteristic predominance of bacteria from the Roseobacter clade (Nogales *et al.*, 2007). We have also observed that bacteria of this group proliferate after artificial pollution with diesel oil (Lanfranconi *et al.* 2010).

**Objectives**

Given these facts, and the evidences relating roseobacters with aromatic hydrocarbon degradation, our aim was to explore the functional diversity of bacteria in polluted harbor waters and waters polluted artificially, with special attention to genes for the catabolism of hydrocarbons, and relate it with the presence of members of the Roseobacter clade.

**Methods**

Eight metagenomes have been generated (20.6 Gb in total) using Illumina technology. Sequences filtered using quality criteria are analyzed using MG-Rast (Meyer *et al.*, 2008), and in house assembly and annotation.

**Conclusions**

Phylogenetic affiliation of sequence reads confirmed the abundance of roseobacters in the metagenomes from polluted samples. Genes coding for peripheral and central pathways for aromatic hydrocarbon degradation have been observed in all metagenomes analyzed. However, at a broad level of resolution we cannot evidence significant differences in number of reads between metagenomes from polluted and control samples. Recruitment of sequence reads against protein databases from Roseobacter genomes and for catabolism of hydrocarbons would help us to define the fine catabolic differences between polluted and unpolluted samples.

Nogales *et al.* (2007) *Environ. Microbiol.* 9:1913-1929; Lanfranconi *et al.* (2010) *Microb. Biotechnol.* 3:607-621; Meyer *et al.* (2008) *BMC Bioinformatics* 9:386.

**FEMS-0984**

**Environmental microbiology**

## **BIOFLOCCULANT PRODUCTION BY ACTINOBACTERIA CONSORTIA AND MEDIA OPTIMIZATION THROUGH RESPONSE SURFACE MODEL**

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### **Background**

Flocculants aggregates suspended particles in solutions thus, reducing turbidity. The industrial application of this process includes water/wastewater treatment.

Conventionally used flocculants have been implicated in deleterious health problems hence, the need for a safe alternative. Bioflocculants are valuable alternative as they are non-toxic and biodegradable.

### **Objectives**

The consortia of *Brachybacterium*, *Cellulomonas* and *Streptomyces* were evaluated for bioflocculant production and their nutritional requirements optimized via response surface model.

### **Methods**

*Cellulomonas*, *Streptomyces* and *Brachybacterium* species were evaluated in pair wise consortium for enhanced production of bioflocculant. Bioflocculant production, flocculation activity testing and media optimization through response surface model were in accordance with standard methods.

### **Conclusions**

Nutritional preferences for bioflocculant production were; glucose (56%;  $2.78 \pm 0.15$  g/l),  $(\text{NH}_4)_2\text{NO}_3$  (53%;  $2.81 \pm 0.37$  g/l) and  $\text{CaSO}_4 \cdot \text{H}_2\text{O}$  (47%;  $2.19 \pm 0.13$  g/l) against *Brachybacterium* and *Streptomyces* species, glucose (92%;  $4.06 \pm 0.62$  g/l), peptone (89%;  $3.88 \pm 0.432$  g/l) and  $\text{MgCl}_2$  (83%;  $3.99 \pm 0.79$  g/l) against *Cellulomonas* and *Brachybacterium* species. Lastly, sucrose (91%;  $4.01 \pm 0.22$  g/L), peptone (82%;  $3.21 \pm 0.13$  g/L) and magnesium chloride (78%;  $3.29 \pm 0.42$  g/L) were preferred by the consortium of *Streptomyces* and *Cellulomonas* species. Plackett-Burman design revealed that the carbon, nitrogen and cation sources were the critical media components essential for the production of bioflocculant. High regression coefficients respectively obtained for the consortia was indicative of the model adequacy ( $P \leq 0.05$ ). The bioflocculant compositional analysis and functional group determination (FTIR spectrometry) indicates a heteropolysaccharides bioflocculant while SEM imaging revealed an amorphous loosely arranged fluffy structure with interstice less than  $1\mu\text{m}$ . The high flocculation activity shown suggests suitability for industrial processes.

**FEMS-1721**

**Environmental microbiology**

**THE INFLUENCE OF AI-2 MOLECULES ON RHAMNOLIPIDS SECRETION  
EXHIBITED BY PSEUDOMONAS AERUGINOSA WITHIN MICROBIAL  
COMMUNITY**

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**Background**

Rhamnolipids are a group of biosurfactants produced by *Pseudomonas* species. It was proven that rhamnolipids participate in biofilm formation and play an important role in microbial motility. Because of their biodegradability and biocompatibility, they are also considered as promising alternatives to widely used synthetic agents in surfactant-mediated bioaugmentation. The cooperation and competition between indigenous and exogenous microorganisms plays an essential role in the final bioaugmentation effect and is based on quorum sensing (QS) mechanism, which enables cell to cell communication. The autoinducers 2 (AI-2) are QS signal molecules observed in both Gram-positive and Gram-negative bacteria. We hypothesize that AI-2 molecules play important role in secretion of rhamnolipids by *Pseudomonas aeruginosa* within microbial community.

**Objectives**

1. To isolate biosurfactant-produced bacteria and investigate the structures of secreted compounds.
2. To investigate the relationship between AI-2 activity and secretion of rhamnolipids.

**Methods**

Isolation: samples originating from cow, chicken and pig feces; screening of bacterial communities by: (i) oil spreading, (ii) drop collapse, (iii) hemolytic activity, (iv) emulsification activity; bioreactor cultivation: Sartorius Stedim, Germany; surface tension: Krüss, Germany; bioluminescence assay: *Vibrio harveyi* BB170 and *Vibrio harveyi* BB152; rhamnolipids identification: HPLC-MS.

## Conclusions

Only monorhamnolipids were detected in the consortium and *Pseudomonas aeruginosa* supernatants. The maximal rhamnolipids production by *Pseudomonas aeruginosa* and AI-2 activity were observed at the same time. The results indicate that QS molecules secreted by associated microorganisms have significant influence on *Pseudomonas aeruginosa* gene expression responsible for rhamnolipids production.

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**FEMS-2628**

**Environmental microbiology**

**THE INFLUENCE OF ZEROVALENT IRON ON THE DEGRADATION OF  
HEXABROMOCYCLODODECANE BY MICROCOSM FROM RIVER SEDIMENT**

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**Background**

Brominated flame retardants (BFRs) have been widely used in many industries over decades. Hexabromocyclododecane (HBCD) is one of the most commonly used BFRs, but it has just listed on the table of persistent organic pollutants (POPs).

**Objectives**

In order to develop cost-effective remediation methods for this emerging pollutant, we evaluated the HBCD degradation ability of one anaerobic microcosm from river sediment, as well as the influence of zerovalent iron (MZVI) on the biodegradation.

**Methods**

An anaerobic sediment sample was acclimated by HBCD. The supernatant solution which contained microcosm was used to conduct further experiments. MZVI (2.5 g L<sup>-1</sup>) was used to degrade HBCD alone or together with the microcosm.

**Conclusions**

The microcosm started to degrade HBCD at day 7, and 30% of HBCD was eliminated in the following 4 days. Sixty percent of HBCD was degraded by MZVI or the co-incubated system after 11 days of incubation. The HBCD biodegradation ability was inhibited by MZVI. MZVI degraded HBCD through reductive debromination, however, this reaction did not occur in the biodegradation setting, suggesting that the microcosm eliminated HBCD through other mechanism. The fluctuation of bacterial community during the degradation processes was monitored. Cluster analysis shown that the microbial composition was less close to that of the MZVI added ones. The inhibition of biodegradation from MZVI may due to the alteration of medium characteristics, which in term changed the microbial composition and function. Our studies provided an insight into the influence of MZVI on the biodegradation of HBCD, which would facilitate the bioremediation of BFRs.





**FEMS-2418**

**Environmental microbiology**

## **BACTERIA ISOLATED FROM DIATOM BLOOMS SEA WATER ADAPT AND GROW IN THE PRESENCE OF POLYUNSATURATED ALDEHYDES**

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### **Background**

Diatoms commonly grow in association with heterotrophic bacteria (Amin et al., 2012) and some species produce polyunsaturated aldehydes (PUAs), which antimicrobial activity (Ianora et al., 2004). Therefore, diatom-associated bacteria may have evolved resistance to PUAs (Ribalet et al., 2008; Balestra et al., 2011), possibly involving adaptive mechanisms of membrane fatty acids (Heipieper and de Bont, 1994; Heipieper et al., 2007).

### **Objectives**

The objectives of this study were the isolation of PUAs-resistant bacterial strains from sea water during diatom blooms and the investigation of bacterial adaptive mechanisms to PUAs.

### **Methods**

Enrichment cultures in mineral medium with PUAs (2*E*,4*E*/*Z*-heptadienal, 2*E*,4*E*/*Z*-octadienal, 2*E*,4*E*/*Z*-decadienal) were inoculated with sea water and incubated at 22°C. PUA-resistant bacteria were isolated, identified by 16S rDNA, grown in the presence of the PUAs and analyzed at membrane fatty acids level.

### **Conclusions**

Six PUAs-resistant isolates, i.e. 1A, 5a, 5b, 7a, 11 and 12b, were assigned to the genera *Pseudomonas*, *Sufflavibacter*, *Halomonas*, *Vibrio*, *Idiomarina* and *Labrenzia*, respectively. Strains 1A, 5b and 7a showed higher growth in the presence of PUAs with respect to the others. Preliminary data on adaptive responses to the PUAs on membrane fatty acid level revealed an increased saturation.

### **References**

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**FEMS-1364**

**Environmental microbiology**

**INVESTIGATION OF BIOTIC AND ABIOTIC FACTORS THAT AFFECT SURVIVAL OF THE PATHOGENIC BACTERIUM *LISTERIA MONOCYTOGENES* IN SOIL**

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**Background**

Agroecosystems are dedicated to the provision of high quality, safe food and sustainability is a major issue. Biological contamination of the food supply raises food safety issues. Prevention of contamination of the food supply requires a thorough understanding of the ecology of pathogenic microorganisms. *Listeria monocytogenes* is a food pathogen found in many habitats including soil, plants, water systems and food processing factories.

**Objectives**

We designed microcosm experiments to decipher extrinsic factors that drive the fate of this model of Human pathogen in soil.

**Methods**

We analysed population dynamics over 80 days after inoculation of a set of 100 soil microcosms. Population dynamics in unsterilised and sterilised soil microcosms were compared. Detailed information such as soil characteristics and land use were integrated into an analysis of variance.

**Conclusions**

The population of *L. monocytogenes* decreased over time but survival depended on the soil under scrutiny. Chemical properties and soil texture affected survival of *L. monocytogenes*. The endogenous soil microbiota had a major impact on the decrease of the population of *L. monocytogenes*.

This prompted us to investigate the relationship between the biodiversity of the soil microbiota and the inhibition of the population of *L. monocytogenes*. We investigated the consequences of soil diversity erosion on the fate of a human bacterial pathogen in the telluric environment. Diversity analysis showed that both soil diversity and phylogenetic composition affected survival of *L. monocytogenes*.

Overall, our results demonstrate that soil biodiversity is a major driver that limit invasion of soil by pathogens.



**FEMS-2126**

**Environmental microbiology**

## **BIOREMEDIATION OF WASTEWATER BY RHIZOBACTERIA AND THEIR APPLICATION IN ARTIFICIAL WETLANDS**

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### **Background**

The treatment of wastewater, before their dumping to the aquatic ecosystems, constitutes a topic of current importance.

### **Objectives**

The objective of the research was to evaluate the physiologic and metabolic diversity of the rhizobacteria from hydrophyte plants of natural wetlands.

### **Methods**

Individual bacterial strains were tested in front of different pollutants. Microbial consortia were faced to water simulating a domestic effluent and synthetic water similar to an industrial effluent. This allowed the selection of the autochthonous strains *Bacillus* sp. (T-119, T-117 and T-229), *Acinetobacter* sp. (T-118), *Pseudomonas* sp. (T-1111) and *Exiguobacterium* sp. (T-316) for their capacities in the elimination of organic matter, ammonium and phosphate. Also, the strains *Bacillus* sp. (T-119, T-117, T-1115 and T-1113) were selected for the lead, chromium and mercury biosorption from synthetic residual waters. Two consortia designed with these strains achieved 75%, 85% and 100% removal of organic matter, ammonium and phosphate and 70%, 9% and 17% of lead, chromium and mercury, respectively. The effect of factors associated to the process of pollutants elimination was determined, which it contributed to the design and bioaugmentation with the consortia of artificial wetlands to laboratory scale. With these systems were reached 100%, over 70% and 55% removal of organic matter, ammonium and phosphate and elimination levels over 95%, 80% and 50% of lead, mercury and chromium, respectively.

### **Conclusions**

It was settled down an integral strategy for obtaining effective bacterial consortia in the bioaugmentation of constructed wetlands, applied in the removal of inorganic and organic pollutants.

**FEMS-1335**

**Environmental microbiology**

## **ON THE EVOLUTION OF WRINKLY SPREADERS, WRINKLEALITY AND FITNESS**

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### **Background**

Bacterial adaptive radiation has been extensively studied using *Pseudomonas fluorescens* SBW25 which gives rise to the Wrinkly Spreader (WS), a class of adaptive mutants producing a wrinkled colony morphology and air-liquid interface biofilms in static microcosms. The WS phenotype is the result of mutations targeting different diguanylate cyclases that up-regulate intracellular levels of c-di-GMP and induce biofilm-formation. Although variations in colony morphology have been noted previously, variations in the WS phenotype (wrinkleality) have not been quantified, nor related to the fitness advantage WS isolates have over other competitors in static microcosms.

### **Objectives**

In this work, we investigated the relationship between wrinkleality and fitness using WS isolates evolved in microcosms containing different growth media.

### **Methods**

Wrinkleality was quantified using a combined biofilm assay (measuring growth, biofilm strength and attachment levels), competitive fitness determined using the non-biofilm-forming reference strain SM-13, and data analyzed by ANOVA and fitness modeled using a GLM approach with environment (media) as the main factor and wrinklealities as covariates.

### **Conclusions**

Significant variation in wrinkleality and fitness was observed between and within WS isolates evolved in different environments. Environment, growth, strength and attachment were all found to have significant effects on fitness. This analyses has shown that growth media selected for subtly different classes of WS, and the underlying differences in phenotype could also help to explain the variation seen in fitnesses. These findings also provide evidence to suggest that WS mutations might have pleiotropic effects and explain why some Wrinkly Spreaders are more successful than others.

**FEMS-1315**

**Environmental microbiology**

**ASSESSMENT OF MICROBIAL CONTAMINATION LEVEL OF SOLID WASTES FROM HOSPITALS IN UNIVERSITY OF PORT HARCOURT, NIGERIA.**

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**Background**

Hospital solid waste disposal has become a major problem in that people indiscriminately dump this waste which pose health danger in the city and therefore there is need to assess these wastes in order to fully understand the micro organisms of health significance which could be transmitted through the improper disposal of these waste materials.

**Objectives**

This study was to evaluate the microbial load of solid health care waste from healthcare institutions in University of Port Harcourt. Solid waste samples were collected randomly from selected units from University of Port Harcourt teaching hospital and LULU Briggs health center

**Methods**

The method adopted was that of Faith Efosa Oviasogie *et al.*,(2010), modified.

**Conclusions**

Bacterial species isolated from the health care solid waste include; *Klebsiella*, *Pseudomonas*, *Staphylococcus*, *Proteus*, *Streptococcus* While *Klebsiella*, *Salmonella* and *Pseudomonas* were isolated from the control samples. The fungal species isolated from the health institutions of University of Port Harcourt include *Asprgillus* and *candida*, where-as *Candida* was isolated from the control sample. The total heterotrophic bacterial count from all samples collected ranged from  $1.8 \times 10^5$  to  $6.8 \times 10^8$  CfU/100g and total fungal count ranged from  $2 \times 10^2$  to  $7 \times 10^4$  CfU/100g. The predominant organisms were *Staphylococcus aureus*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Some of the bacterial isolates have been incriminated in nosocomial infections.

**FEMS-1269**

**Environmental microbiology**

## **FRESHWATER ANAEROBIC OXIDATION OF METHANE ASSOCIATED WITH SULFATE REDUCTION IN A NATURAL GAS SOURCE**

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### **Background**

Anaerobic oxidation of methane (AOM) coupled to sulfate reduction (SR) occurs in marine sediments and the responsible microorganisms are communities of anaerobic methanotrophic archaea (ANME) and sulfate reducing bacteria (SRB) of the SEEP-SRB1 clade. AOM coupled to SR is rarely documented in terrestrial environments where sulfate concentrations are generally low.

### **Objectives**

The possible occurrence of AOM coupled to SR was investigated in a natural freshwater gas source. This was done to know where and if AOM coupled to SR occurs in terrestrial environments and which organisms execute this process.

### **Methods**

<sup>13</sup>CO<sub>2</sub> formation and methanogenesis was monitored at long term (>168 days) in slurries incubated with <sup>13</sup>CH<sub>4</sub> and different electron acceptors (sulfate, nitrate, ferrihydrite, humic acids). <sup>13</sup>CH<sub>4</sub>, <sup>12</sup>CH<sub>4</sub> (produced during methanogenesis) and <sup>13</sup>CO<sub>2</sub> were quantified using gas chromatography coupled to mass spectrometry. Electron acceptors were analyzed by Ion Chromatography and iron and sulfide by colorimetric methods. Microbial community analysis was done using 16S rRNA pyrosequencing and quantification of specific microbial groups was done using quantitative PCR.

### **Conclusions**

Slurry incubations gave evidence that AOM occurred only with sulfate as electron acceptor. Sulfide production occurred simultaneously with <sup>13</sup>CO<sub>2</sub> production and no methanogenesis occurred. Pyrosequencing analysis showed presence of ANME-2a/b and ANME-2d 16S rRNA genes. Higher abundance of ANME2a/b in incubations with methane and sulfate was confirmed with qPCR analysis. Bacterial pyrosequencing showed presence of SRB sequences belonging to SEEP-SRB1. This is the first report



that unequivocally shows that AOM is associated with SR in a freshwater environment.

**FEMS-0516**

**Environmental microbiology**

**RHIZOSPHERIC MICROBIAL FLORA OF THE INDIAN ECOTYPE PTERIS VITTATA MEDIATING ARSENIC PHYTOREMEDIATION**

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**Background**

Mitigation of arsenic (As) pollution is a topical environmental issue of high R&D priority. *Pteris vittata* is a proven arsenic hyperaccumulator and has capability of remediating arsenic from water and soil through phytoextraction. There are a few reports on rhizospheric microflora of *Pteris vittata* which could probably mediate As uptake by the plants, but there are no reports of such study on this Indian ecotype of *Pteris vittata* identified by us.

**Objectives**

This investigation was carried out to isolate and study role of rhizospheric microbes of the Indian ecotype *Pteris vittata*.

**Methods**

A glasshouse experiment was conducted to study rhizosphere microbes of *P.vittata* grown in soil amended with 25 mg arsenic Kg<sup>-1</sup> soil in the form of sodium arsenate for sixty days. A total of 12 bacteria cultures were isolated and marked as As1-As12 and they were further studied for Minimum Inhibitory Concentration, Arsenic transformation test and Plant Growth Promoting Characteristics (PGPRs) i.e. Siderophore, IAA and ACC deaminase.

**Conclusions**

The isolated microbes showed arsenic tolerance up to 1000ppm and have dual capacity of AsV reduction and AsIII oxidation. Out of the twelve isolates, As 1, 2, 11, 12 exhibited siderophore positive. Except As 3, 4, 5, 9 & 12, all produced IAA while ACC deaminase was absent in all isolates. These findings show the interaction between plant and microbes for As detoxification and remediation by this ecotype of *P.vittata* endogenous to India. Our study opens up further scope of using the isolated arsenic tolerant microbes for enhancing the As remediation process through phytoremediation.

**FEMS-1265**

**Environmental microbiology**

**EFFECT OF ATTENDANCE ON THE SEDIMENT MICROBIAL COMMUNITIES OF 14 SANDSTONE CAVES IN THE PROTECTED LANDSCAPE AREA LABSKÉ PÍSKOVCE (CZECH REPUBLIC)**

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**Background**

In the Protected Landscape Area Labské pískovce (Czech Republic) there is a series of sandstone caves accessible with climbing equipment prevailing; their access is subjected to permission and attendance recorded in the log-books.

**Objectives**

Investigation of the effect of attendance on the quantity, rough composition, stress indicators, and activities of microbial communities in cave-sediments in 14 caves using number of visitors and step -compression as the main tested factors.

**Methods**

Microbial communities were characterized by phospholipid fatty acid (PLFA) profiles and activities of extracellular oxidases, peroxidases, phosphatases, proteases, and glukosidases.

**Conclusions**

Sediment microbial communities were generally poor (low PLFA content, low activities). Step-compression increased significantly ratios of fungal/bacterial and G+/G- PLFA as well as PLFA of actinobacteria and activities of phosphatases, glukosidases, and peroxidases while decreased *trans/cis* PLFA indicator of microbial

stress. Number of visitors correlated positively with G+/G- ratio and negatively with soil moisture, *trans/cis* PLFA stress indicator, and activity of peroxidases. Overall the effect of attendance to sediment microbial communities was found significant and it is likely related to input of nutrients into caves. The results might serve as a supporting material for reconsidering of the cave attendance policy.

**FEMS-1092**

**Environmental microbiology**

## **STUDY OF NEMATODE-BACTERIA INTERACTION IN PINE WILT DISEASE**

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### **Background**

Pine wilt disease (PWD) is one of the most threatening diseases for worldwide forestlands, and is caused by the plant parasitic nematode *Bursaphelenchus xylophilus*. Bacterial communities associated with the *B. xylophilus* are suggested to play a role in PWD development.

### **Objectives**

Our aim is to understand the contribution of nematode-associated bacteria (in particular, *Serratia quinovorans* FX1 and *Serratia marcescens* PWN146) in PWD, as well as their behavior towards *B. xylophilus*.

### **Methods**

Previously, we have characterized the phenotype of *S. quinovorans* FX1 and *S. marcescens* PWN146, and their performance under *in vitro* oxidative stress conditions, alone and in association with *B. xylophilus*. We are now analyzing both genomes and comparing both with endophytic and plant pathogenic bacteria.

### **Conclusions**

Both *Serratias* present phenotypic plasticity in traits that favour their fitness inside of the host tree (Vicente et al. 2011; Vicente et al., 2012), and under certain circumstances can help the nematode to overcome the hostile tree environment (Vicente et al., 2013). We believe that the beneficial association nematode-bacteria may be facultative and co-dependent of other unknown features (Nascimento et al. 2014).

Vicente et al. 2012, PlosOne 7 e46661; Vicente et al. 2013, BMC Microbiology 13: 299; Nascimento et al. 2014, Environmental Microbiology Reports 08



**FEMS-1164**

**Environmental microbiology**

## **DIVERSITY AND BIOTECHNOLOGICAL POTENTIAL OF THRAUSTOCHYTRIDS FROM THE CHINA'S COASTAL WATERS**

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### **Background**

Thraustochytrids are unicellular and heterotrophic fungal-like marine protists with known low diversity and have long been known to produce diverse interesting bio-products. However, their diversity and biotechnological potential have been barely explored in the china's coastal waters.

### **Objectives**

Investigate the diversity of thraustochytrids using molecular and cultivation methods; explore the isolated strain in the production of biotechnological potential.

### **Methods**

Flow Cytometer, pyrosequencing, plate cultivation, fermentation, and HPLC analysis

### **Conclusions**

Thraustochytrids abundance displays significant spatial and temporal variations in the coastal waters and can surpass that of bacteroplankton. The molecular diversity of thraustochytrids is much higher than what we have known. Samples from contaminated areas tend to contain higher richness and diversity than those uncontaminated areas. The majority of recovered reads are members of the family Thraustochytriaceae. Thus, thraustochytrids were the dominant components of labyrinthulomycetes and presumably play significant role ocean carbon cycling the coastal waters. The culturable diversity of thraustochytrids is low in coastal waters. However, most of cultivated strains produces carotenoids, polysaccharides and proteases. Some of the stains produce significant amount polyunsaturated fatty acids (PUFA) (e.g. DHA) and saturated fatty acids. Thus, thraustochytrids can be used for the production nutraceuticals and biodiesel.

**FEMS-2812**

**Environmental microbiology**

## **BIOCHAR COLONIZATION BY ANAEROBIC MICROORGANISMS**

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Background: Terra preta do indio is a typical hortic anthrosol in the amazonas region derived from long-term human landscape cultivation. This distinct type of soil is characterized by a high storage capacity of plant nutrients as well as a high content of carbon enabling agriculture even on soils with low humus and/or nutrient content. Due to certain similarities in consistence and composition, biochar is recently discussed as a value artificial soil amendment to obtain similar positive effects on soil fertility like terra preta. In addition to the structural and compositional analysis of biochars the interaction of the chars with living microorganisms is studied, to explain the effect of biochar enriched soils from a microbiological point of view. Due to seclusion of a biogas system and the consequent creation of a separate world the study of effects by biochar colonization with microorganisms by minimizing the confounding factors is more easier compared with soil tries.

Objectives: In this study the microbial interaction caused by colonization, biofilm formation and degradation with two types of char (hydrochar and pyrolyzed char), which differs in degradation stability, is examined using a mesophilic gas lift biogas fermentation system.

Methods: In samples taken from mesophilic to thermophilic biogas system at various time points the bacteria:archaea ratio was determined by DNA-based quantitative PCR according to Yu et al. [1995].. In addition, the treated chars were investigated with respect to their surface properties by gas adsorption method (BET).

Conclusions: Caused by feedstock and process condition during carbonization the pyro-char shows higher stability regarding degradability while fermentation. This leads due to the colonization of the char to a measurable reduction of pore surfaces in the pyro-chars, whereas the instability of the hydrochars lead to an increase in the specific surface area compared with the unfermented sample. Most interesting is the dynamic not stable population in the microbial community over time under anaerobic digestive conditions.



**FEMS-2775**

**Environmental microbiology**

**COMPARATIVE ANALYSIS OF THE MICROBIAL COMMUNITIES ON THE SURFACE OF MINERAL ENHANCED BIOCHARS**

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**Background**

Biochar is a kind of porous material made by the pyrolysis of biomass under complete or partial exclusion of oxygen. It has applications in different areas, with the most important one being as an amendment to improve the quality of agricultural soils. Several studies reported significant changes for the microbial communities in bulk soil with biochar-treatment, whereas microbiota colonised on the surface of biochar is largely neglected.

**Objectives**

In this study, two types of mineral enhanced biochars, including kaolinite (Kao) and bentonite (Be), and non-enhanced biochar (Bam) were applied into soil with (F) or without (NF) traditional fertilisation (urea, superphosphate and potash) on a pot trial. We compared the differences of microbial communities on the surface of biochar and amended soil after 4-month incubation.

**Methods**

Total DNA was extracted from biochar particles and soil respectively and sequencing using the Illumina MiSeq platform.

**Conclusions**

Analysis of 2001281 quality-filtered sequences revealed high microbial diversity in these samples. Bray-Curtis distance-based non-metric multidimensional scaling (nMDS) analysis showed that the microbiota of biochar particle and soil were clearly separated into four clusters (soil vs. particle, F vs. NF). Samples in the NF cluster were observed to vary by biochar types. *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Proteobacteria* was detected as the dominant phyla regardless of particle and soil. Furthermore, unique OTUs in each samples were identified, three OTUs from the class *Gammaproteobacteria*, the family *Oxalobacteraceae*, the genus *Thiobacillus* were found significantly more abundant in biochar particles.

**FEMS-2499**

**General virology and bacteriophages**

## **CHARACTERIZATION OF ENDOLYSINS FROM A VIBRIO PARAHAEMOLYTICUS BACTERIOPHAGE VPP-KF1**

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### **Background**

*Vibrio parahaemolyticus* is a halophilic gram-negative bacterium in seawater and estuaries. It is one of the leading causes of seafood-related illness through the ingestion of raw or undercooked seafood and cross contamination from unsanitary cooking environments. Endolysin, a phage-encoded enzyme that breaks down bacterial peptidoglycan at the terminal stage of the phage reproduction cycle, is reported to be effective for the control of pathogenic bacteria as well as bacteriophage itself.

### **Objectives**

The purpose of this study was to examine lytic properties of endolysins from bacteriophage Vpp-KF1 infecting *Vibrio parahaemolyticus*.

### **Methods**

Lytic bacteriophage Vpp-KF1 infecting *Vibrio parahaemolyticus* was isolated from west coastal area of Korea, and its whole genome was sequenced and analyzed. Endolysin genes were identified in the bacteriophage genome, and then the endolysin genes were cloned and overexpressed in *Escherichia coli*. The endolysins were purified by Ni-NTA affinity chromatography. The effects of temperature and pH on the lytic activities, and the lytic spectrums of endolysins were determined by measuring changes in the optical density of cell suspension.

### **Conclusions**

Endolysin genes were successfully expressed in *E.coli* expression system, and the enzymes were purified as soluble forms. The endolysins were relatively stable and active over broad temperature (from 24°C to 55°C) and pH (from 2 to 10) ranges. The lytic spectrums were relatively narrow because those endolysins were effective mainly in *Vibrio* species when treated without EDTA. These results suggested that the bacteriophage Vpp-KF1 endolysins have potential as an alternative to antibiotics or antibacterial agents in controlling bacterial contamination.



## FEMS-1275

### General virology and bacteriophages

#### THE METABOLIC EFFECTS OF PHAGE INFECTION: PHAGE SPECIFIC OR NOT?

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#### Background

Bacterial viruses are fully dependent on their host cell metabolic resources to fuel their propagation. Through millions of years of co-evolution, many bacteriophages have acquired host genes in their genomes which can overcome biochemical bottlenecks in specific metabolic processes, and therefore were termed 'auxiliary metabolic genes' (Thompson *et al*, 2011).

#### Objectives

As such, the metabolic response to phage infection would vary depending on the number of AMG's in the genome of the phage. To gain additional insight in this topic, we studied the metabolic variations in an ubiquitous, gram-negative bacterium, *Pseudomonas aeruginosa*, under attack of six distinct lytic dsDNA phages.

#### Methods

We used state of the art metabolomics techniques (Fuhrer *et al*, 2011). A total of 6006 ions were measured, of which 918 were annotated to *P. aeruginosa* metabolites. After filtering 375 unique masses were retained, corresponding to 810 *P. aeruginosa* metabolites.

#### Conclusions

Using hierarchical clustering we saw that the metabolic response triggered by phage infection is not a general response but depends on the requirements of a specific phage. A pathway enrichment analysis showed that not all pathways were influenced equally. An example is the clear up regulation of the pyrimidine metabolism, which can be linked to a study from Enav *et al* in 2014 that showed that AMG's with a function in the pyrimidine and purine metabolism are enriched in the viral gene pool. These results indicate that unique AMG's might play a crucial role in the specific host metabolic response induced by different phages.

**FEMS-0726**

**General virology and bacteriophages**

**LACTOCOCCUS GARVIEAE'S VIRUSES: AN INSIGHT INTO TEMPERATE AND VIRULENT BACTERIOPHAGES**

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**Background**

*Lactococcus garvieae*, one of the most important pathogens in the aquaculture sector, has been recently recognized as an opportunistic human pathogen. However, little is known about the factors controlling of its growth.

**Objectives**

The aim of this project is to isolate and characterize temperate and virulent phages of *L. garvieae*.

**Methods**

Forty-five *L. garvieae* strains isolated from various ecological niches were tested for the presence of inducible prophages. Virulent phages were also isolated from different environments. Morphological and genomic characteristics of the new phages were studied through transmission electronic microscope (TEM), DNA restriction profile, protein profile, host range, and in few cases, genomic sequences.

**Conclusions**

Twenty temperate phages belonging to the *Siphoviridae* family and five new virulent phages able to infect *L. garvieae* strains have been isolated and partially characterized. Temperate phages, integrated into the genome of the host cell, promoted genome plasticity and appeared to be involved in gene mobilization. Virulent and strain-specific phages may be useful as alternatives to antibiotics to treat *L. garvieae* infections.

**FEMS-1462**

**General virology and bacteriophages**

### **ANALYSIS OF CDT-I PHAGE PRODUCTION IN ESCHERICHIA COLI**

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#### **Background**

Cytotoxic distending toxin (CDT)-producing *Escherichia coli* (CTEC) have been isolated from patients with diarrhea. Previously, we have reported that *cdt-I* genes in *E. coli* are located in genetically diverse lambdoid phages, and CDT-I phage (CDT-I $\Phi$ ) could be transferred horizontally between *E. coli* in pathotype-specific manner.

#### **Objectives**

In this study, CDT-I $\Phi$  was comprehensively analyzed to understand underlying mechanisms of its production.

#### **Methods**

Culture supernatants of 46 CTETC-I strains were examined for CDT-I $\Phi$  production by plaque assay with *E. coli* C600 both in the presence and absence of mitomycinC (MMC). Plaque assay was also performed with *DrecA-E. coli* (strain TOP10F') and its *recA* complement as a recipient strain. Presence of other lambdoid phages was examined by using 23 known *cI* gene-probes.

#### **Conclusions**

Among analyzed strains belonging to diverse EPEC and non-EPEC, only EPEC O127 and O142 could produce CDT-I $\Phi$  plaques. All the CTETC-I O142 produced CDT-I $\Phi$  both in the presence and absence of MMC, whereas CTETC-I O127 produced only in the presence of MMC. However, under identical host background (C600), these phenomena disappeared. CDT-I $\Phi$  plaque was not produced against *DrecA-E. coli*, whereas produced when complemented with *recA* gene. CTETC-I O127 strains harbored 6 or 7 *cI* genes, whereas O142 had 2~4. CDT-I $\Phi$  production was differently regulated in CTETC-I O127 and O142, suggesting involvement of host factors, which might contribute to inhibit or stimulate spontaneous CDT-I $\Phi$  induction in these serogroups in a RecA-dependent pathway. Moreover, intra-prophage interactions with O127-specific prophages could be related to CDT-I $\Phi$  production.

**FEMS-2712**

**General virology and bacteriophages**

**BACTERIOPHAGES ISOLATED FOR THE CONTROL OF PATHOGENIC VIBRIO HARVEYI IN SHRIMP HATCHERIES**

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Phage therapy has been accepted as alternative to chemotherapy in aquaculture systems especially in shrimp larval production. Uniqueness of bacteriophages as therapeutics is their self-replicating character and specificity to the target organisms. However, concerns exist with their use in vivo as they may turn out to be the agents of generalized transduction in the environment mediating transfer of genes among bacteria. This is important because phages selected for therapeutic purpose must not carry virulence genes or properties conferring antimicrobial resistance and toxin genes to the host bacteria. Moreover, therapeutic phages should not become latent as well during their life cycle. Using an enrichment method, bacteriophages were isolated from sediment and water samples from Vembanad estuary, Cochin, India employing 12 isolates of *Vibrio harveyi*. By this method four phage lysates were obtained, purified by soft agar overlay method, titred and stocked at 4° C and stability checked over a year. Host range of the phage was assessed against 162 bacterial isolates spanning 14 species to which they showed broad spectrum lytic activity. One phage was lytic to both *V. alginolyticus* and *V.*

*parahaemolyticus* potentially pathogenic to shrimp and other aquatic animals in culture systems. Lytic efficiency of all phages was checked on their respective host bacterial species. In all cases the phages were able to arrest growth of *V. harveyi* for about 5-6 hours after which phage resistant forms emerged and began to dominate. By electron microscopy, three of the phages were identified as Myovirus-like with icosahedral head, short collar and contractile tail. The fourth one was Siphovirus-like with icosahedral head and long, flexible, helical non-contractile tail. It was infective to 62 % *V. harveyi* isolates tested and to its near taxonomical neighbours, *V. alginolyticus* and *V. parahaemolyticus*. Development of resistance or lysogenization is an impediment in the development of phage therapy in aquaculture necessitating deeper understanding on their genome and phage-host dynamics in the environment.

**FEMS-1184**

**General virology and bacteriophages**

## **HIGH FREQUENCY OF MUTATOR STRAINS INDUCED BY PHAGE GHOST ATTACHMENT**

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### **Background**

Lytic phages induce bacterial mortality and contribute to the global carbon cycle. Because their main impact on the host is lysis of the cell, short-term contact between the host and phage is considered genetically less important unless genetic exchange occurs.

### **Objectives**

In order to understand the genetic impact of a lytic phage on the host, we examined the fate of host cells after attack by a lytic phage and the mutant frequency of cells that did not undergo lysis.

### **Methods**

After incubating *Escherichia coli* with phage T4 or the ghost for 10 min, the cells were placed on LB plates and allowed to grow. Colonies that escaped lysis were randomly selected. Mutant frequency to nalidixic acid resistance in the escaped colonies was examined. The insertion of phage DNA and mutations on the genomes of mutator strains which showed elevated mutant frequencies were examined by whole genome sequencing analysis. Role of DNA polymerase IV gene (*dinB*) was examined with RT-PCR and *dinB*-deficient mutant.

### **Conclusions**

After the attachment of the phage T4 to *E. coli* cells, 1% *E. coli* cells showed an approximately 40-fold increase in mutant frequency. Phage ghosts increased the incidence of mutator strains. Phage DNA was absent in the mutator strains. The attachment of the phage ghosts to the host induced the mRNA expression of the *dinB*, resulting host genome mutagenesis. These results suggested that attachment of a lytic phage alters the genome of the host that has escaped lysis at high frequency, without relying on gene transfer.



**FEMS-2500**

**General virology and bacteriophages**

## **TACMAN-BASED REAL-TIME PCR FOR QUANTITATIVE DETECTION OF VIRAL HEMORRHAGIC SEPTICEMIA VIRUS**

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### **Background**

Viral hemorrhagic septicemia virus (VHSV) is known as the most common cause of diseases in fisheries. VHSV infects fishes of fresh water as well as ocean. Natural VHSV may cause of disease by introducing into fish farm. A huge amount of fry are stocked into the coast.

### **Objectives**

Therefore development of VHSV detection method is required. For the detection of VHSV, enzyme immunoassay using polyclonal antibodies, western blotting, and electron microscopy are mainly used. However these methods have limitation to detect latent infection and an initial viral disease. Recently nucleotide amplification test using polymerase chain reaction (PCR) has been used widely for rapid and specific detection of viruses.

### **Methods**

In this study, TaqMan-based real-time PCR assays to detect VHSV was developed and validated. Specific primers for amplification of VHSV were selected, and VHSV was quantified by use of TaqMan probe.

### **Conclusions**

The sensitivity for VHSV real-time PCR was calculated to be  $8.10 \times 10^{-1}$  TCID<sub>50</sub>/mL. The TaqMan probe real-time PCR assay was proven to be reproducible and very specific to VHSV. Therefore, it was concluded that this rapid, specific, and robust assay could replace the conventional assay for detection of VHSV.

**FEMS-2655**

**General virology and bacteriophages**

## **COMPARATIVE ANALYSIS OF AVIAN PATHOGENIC ESCHERICHIA COLI PHAGES**

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### **Background**

Avian pathogenic *Escherichia coli* (APEC) causes colibacillosis and significant economic losses in the poultry industry.

### **Objectives**

To isolate and characterize poultry fecal phages that are active against *Escherichia coli* serogroups O1, O2, and O78; the main causative agents of avian colibacillosis.

### **Methods**

Lytic phages (71) were isolated from poultry fecal material and their lytic spectrum of activity was determined against 61 APEC (O1, O2, O78 serotypes) strains collected from Belgium. The phages with the broadest activity spectrum were characterized by, restriction-fragment length polymorphism (RFLP). Based on their obligate lytic characteristics and broad host spectrum of activity, some were selected for whole genome sequencing using the Nextera XT™ kit (Illumina, San Diego, USA). The morphology of the phages was determined by negative staining and Transmission Electron Microscopy (TEM).

### **Conclusions**

Phages PhK7, PhK10S, PhK10L, PhK12B, PhK21, PhK22, PhK12A, PhK13, PhK26, PhK2, PhK3, PhK6, Ph431, PhC3 and PhK33C were isolated and further characterized. The most active phages were Ph431 active against 70% (43/61) of the APEC strains, PhC3 against 68% (42/61), PhK33C against 41% (25/61), PhK10S against 26% (16/61), Ph12A 19% against (12/61).

In all samples, particles were observed consisting of a non-enveloped head with icosahedral symmetry and a tail with helical symmetry; characteristics attributing the particles to the bacteriophage order of the Caudovirales and belonged to families Myoviridae and Siphoviridae.

Genome analysis of some of phages indicated there were close relatives of Siphoviridae present under the unclassified Tunalikevirus (PhK2, PhK3, PhK6) and unclassified Siphoviridae (PhK10S, PhK7, PhK10L, PhK12B, PhK21, PhK22, Ph12A, PhK26, PhK13).

**FEMS-2503**

**General virology and bacteriophages**

**OBSERVATION OF PHAGE-ANTIBIOTIC SYNERGY IN VARIOUS COMBINATIONS OF BACTERIA, BACTERIOPHAGE, AND ANTIBIOTICS**

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**Background**

When phages infect bacteria cultured in the presence of sub-lethal doses of antibiotics, sizes of phage plaques significantly increase. This phenomenon is known as phage-antibiotic synergy (PAS).

**Objectives**

We extended observation of PAS to a wide variety of bacteria-phage pairs with four different classes of antibiotics.

**Methods**

In the presence of sublethal dose of various antibiotics, phage growth and burst was measured.

**Conclusions**

PAS was observed in both Gram-positive and Gram-negative bacteria. Cell wall synthesis inhibitors and DNA metabolism inhibitors generally induced PAS. Some protein synthesis inhibitors induced PAS, while others did not. The use of sub-lethal dose of ampicillin, cefotaxime, ciprofloxacin, or mitomycin C allowed formation of highly visible plaques of increased sizes when various bacteriophages infected Gram-positive bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, and *Enterococcus faecalis*. In Gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa* phages showed increased plaques in the presence of sub-lethal dose of cefotaxime, ciprofloxacin, or mitomycin C. We also confirmed that cells stressed with  $\beta$ -lactam and quinolone antibiotics filamented or swelled extensively. Burst sizes of bacteriophages also increased in the presence of antibiotics. Increase of production of phages in the presence of antibiotics was also shown in vivo using *Caenorhabditis elegans* as a model animal. Induction of SOS response was observed in many cases of PAS, but not all. Enlarged production facility allowed increased DNA replication, transcription, and translation of phage leading to PAS.

**FEMS-1642**

**General virology and bacteriophages**

**IMMUNOGENICITY EVALUATION OF HEPATITIS B (HB) VACCINES UTILIZED IN THE EXPANDED PROGRAMME ON IMMUNIZATION (EPI) IN SOUTH-EASTERN NIGERIA**

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**Background**

Vaccination effectively enables the control of many infectious diseases. However, we cannot always avoid the problem of adverse reactions accompanied by vaccination. The most common cause of vaccine-related side-effects is human errors linked to logistics problems.

**Objectives**

This study investigated

- whether or not the Hepatitis B (HB) Vaccines in use in South-East Nigeria have the features and characteristics needed for them to satisfy stated or implied needs.
- the efficiency of cold-chain facilities in South-east, Nigeria

**Methods**

The immunogenic quality of the vaccines was determined by quantitation of the antibody titer (using ELISA method) in vaccinated laboratory animals.

The mean antibody titers for the control, Enugu/Ebonyi, Imo, Anambra and Abia States' vaccines were 8.7, 398.2, 353.7, 396.3 and 369.6 mIU/mL respectively. The vaccine used as the control did not produce sufficient protective effect (antibody titer was less than 10 mIU/mL). One-way analysis of variance shows that the means of all the samples, including the control, are significantly different at  $P < 0.05$ . Also, Bartlett's test for equal variances showed that the variances are also significantly different. The vaccines do not all produce equal protection ( $P$  value was  $< 0.0001$ ), their antibody titers being statistically different. The Dunnett's Multiple Comparison Test shows that the mean antibody titers produced by the vaccines from the States significantly differ from the value produced by the control vaccine at  $P < 0.05$ .

**Conclusions**

All the vaccines tested produced the required amount of protective antibodies. The cold-chain facilities, at the time of vaccines collection, are in good condition.

**FEMS-0692**

**General virology and bacteriophages**

**BACTERIOPHAGE ENDOLYSIN LYT  $\mu$ 1/6: BINDING ACTIVITY REVEALED!**

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**Background:** Bacteriophage endolysins are phage-encoded peptidoglycan hydrolases by which bacteriophage are released from bacterial cells and which are effective in bacterial cell wall lysis, when applied exogenously. The gene product of orf50 from actinophage  $\mu$ 1/6 of *Streptomyces aureofaciens*, producing tetracycline, is endolysin Lyt  $\mu$ 1/6. It shows a two-domain modular structure, consisting of an N-terminal catalytic and a C-terminal binding domain (CBD). BLAST analysis of Lyt  $\mu$ 1/6 CBD disclosed the presence of a peptidoglycan binding domain type 1 (PG\_binding\_1) which was also confirmed by *in vitro* experiments.

**Objectives:** The main aim of this study was to determine the part of the Lyt  $\mu$ 1/6 CBD responsible for binding activity towards the *Streptomyces* cell wall, as well as to study concrete truncated forms of it in fusion with green fluorescent protein (GFP).

**Methods:** The sequence of the Lyt  $\mu$ 1/6 CBD and truncated forms of it were cloned and expressed in *Escherichia coli*. All recombinant proteins were isolated and partially purified with conventional chromatographic methods. The ability of the CBD truncated variants fused to GFP to bind to the substrate consisting of *Streptomyces aureofaciens* NMU cells was demonstrated by specific binding assays. Subsequently, the binding of the CBD fusion products was visualized by fluorescence microscopy.

**Conclusions:** It could be assumed that the binding activity of the CBD is located within the three helices of the PG\_binding\_1 domain. This fact is supported by a negative control which is a construct of the N-terminal part of the Lyt  $\mu$ 1/6 CBD, showing no binding activity.

**Keywords:** bacteriophage endolysin, *Streptomyces aureofaciens*, cell wall binding domain (CBD), green fluorescent protein (GFP), truncations, binding activity

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**FEMS-1830**

**Global regulatory networks**

**THE ROLE OF AVIN08930 IN BIOSYNTHESIS OF POLY-B-HYDROXYBUTIRATE AND ALKYLRESOLCINOLS IN AZOTOBACTER VINELANDII**

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**Background**

*Azotobacter vinelandii* is a Gram-negative bacterium able to synthesize polyhydroxybutyrate (PHB), a polyester to produce biodegradable plastic. *A. vinelandii* can undergo a differentiation process to produce resistant-desiccation cysts. During the encystment, the bacterium synthesizes the phenolic lipids alkylresorcinols (ARs), which replace the membrane phospholipids. The operons *phbBAC* and *arsABCD* encode the enzymes necessary for PHB and ARs synthesis and their transcription are activated by PhbR and ArpR, respectively.

The two component regulatory system GacS/GacA is essential for biosynthesis of PHB and ARs, since a *gacA* mutation abrogates their production. GacA activates the transcription of genes encoding sRNAs *rsmZ1-7* and *rsmY*, which bind to RsmA; a small protein that blocks the translation of *phbR* and *arpR* mRNAs. Because inactivation of *rsmA* in a *gacA* mutant did not restore the PHB and ARs synthesis, we hypothesize that there is another pathway by which GacS/GacA controls the biosynthesis of PHB and ARs in *A. vinelandii*.

**Objectives**

To characterize the role of Avin08930 on PHB and ARs in *A. vinelandii*.

**Methods**

A bioinformatic analysis of *A. vinelandii* genome allowed us to identify a consensus binding site for GacA in the Avin08930 promoter. By using qPCR and transcriptional fusions, we determined if Avin08930 expression is dependent of GacA. An Avin08930 mutant was constructed and PHB and ARs synthesis as well as *phbB* and *arsA* expression were determined in this strain.

**Conclusions**

The transcription of Avin08930 is activated by GacA. In the Avin08930 mutant the *phbB* and *arsA* expression as well as the PHB and ARs levels were reduced.





**FEMS-1736**

**Global regulatory networks**

## **A POTENTIAL NOVEL GENE TRANSCRIPTION REGULATORY SYSTEM IN STAPHYLOCOCCUS GENUS**

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### **Background**

Gene transcription regulatory pathways are crucial for adaptation to constantly-changing environmental conditions. These include pathogen-host interactions. Up until now, few such systems have been characterised for *Staphylococcus aureus* and simultaneously demonstrated as relevant for virulence.

### **Objectives**

This study presents a comprehensive analysis of a three-gene operon *saoABC*, which likely constitutes yet uncharacterised staphylococci-specific gene expression regulatory system. The research is focused on the operon structure and conservation among species of *Staphylococcus* genus, DNA-binding properties of SaoC protein, changes in *saoABC* operon genes expression triggered by stress stimuli, links between *saoABC* operon and known gene transcription regulatory systems as well as on differences between phenotypes of the wild type and the null mutants of *saoA* and *saoC* genes.

### **Methods**

The applied methods include comparative genomic analysis, heterologous SaoC protein expression and its purification, Real-Time PCR gene expression quantification, allelic replacement, virulence analysis in chicken embryo model and intracellular survival within human gingival fibroblasts and keratinocytes.

### **Conclusions**

The revealed features of *saoABC* operon convincingly suggest that it is a novel operon of a transcription regulation system, likely of a yet uncharacterised alternative sigma factor. The changes in the operon genes expression specifically induced by starvation and acidification point out the possible role of *saoABC* in intracellular survival of staphylococci in host cells.

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**FEMS-2516**

**Global regulatory networks**

**TRANSCRIPTIONAL CONTROL MEDIATED BY THE CELL CYCLE REGULATOR GCRA AND CHROMOSOME METHYLATION IN CAULOBACTER CRESCENTUS**

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**Background**

In *Caulobacter crescentus* cell cycle is controlled at multiple levels by a plethora of regulators, such as GcrA, a putative transcriptional regulator with no functional characterization. Another regulator involved in controlling gene expression is CcrM, a GAnTC-specific N6-adenosine methylase active only at the end of S-phase; as a consequence, at every round of replication a fully methylated promoter of a gene gives two different hemi-methylated copies.

**Objectives**

Here we investigated *in vivo* and *in vitro* the role of GcrA during the S-phase on the transcription of important cell cycle genes that are controlled by CcrM.

**Methods**

We used a combination of ChIP (chromatin-immunoprecipitation), biochemical and biophysical experimentation and genetics to show that GcrA is a dimeric DNA-binding protein that preferentially targets promoters harbouring CcrM methylation sites in *Alphaproteobacteria*. After tracing CcrM-dependent promoter marks at a genome-wide scale, we showed that these marks recruit GcrA *in vitro* and *in vivo*. Moreover in the presence of a methylated target, GcrA recruits the RNA polymerase to promoters, consistent with its role in transcriptional activation. *In vitro* transcription assay using purified GcrA and RNAP on several GcrA-regulated genes along the chromosome showed that GcrA is a modulator of transcription that activates or represses depending on the methylation state of promoters. We confirmed this observation *in vivo* using RNA-FISH, revealing in addition a potential role of GcrA in spatial regulation of transcription.

**Conclusions**

In conclusion from our results we propose a novel model of methylation-dependent transcriptional regulation that modulates the expression of important genes during cell cycle.

**FEMS-0762**

**Global regulatory networks**

## **CROSS-REGULATION OF MULTIPLE DISPARATE GENE CLUSTERS BY PAS-LUXR TRANSCRIPTIONAL REGULATORS**

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### **Background**

PAS-LuxR regulators are highly conserved proteins devoted to the control of antifungal production by binding to operators located in given promoters of polyene biosynthetic genes. These regulators have been found to be encoded in all know biosynthetic gene clusters of polyene polyketides, and hence considered pathway-specific modulators.

### **Objectives**

The canonical operator of PimM, archetype of this class of regulators, has been used to search for putative targets of orthologous protein PteF in the genome of *Streptomyces avermitilis*.

### **Methods**

We have used bioinformatic and electrophoretic mobility shift techniques to identify PimM binding sites along the genome, and mutants in *pteF* for the functional characterization of selected operators.

### **Conclusions**

Contrary to the stablished opinion, PAS-LuxR regulators may control directly many different processes previously unforeseen, such as genetic information processing, DNA replication and repair, energy, carbohydrate, and lipid metabolism, morphological differentiation, transcriptional regulation, and other secondary metabolites biosynthesis. To prove that, we demonstrate that PteF, is able to cross-regulate the biosynthesis of the ATP-synthase inhibitor oligomycin in addition to that its canonical target filipin. Our results place these regulators as wide-domain modulators in regulation networks, and open new possibilities for the enhancement and awakening of metabolite production in *Streptomyces*.



**FEMS-3053**

**Global regulatory networks**

**TRANSCRIPTOMIC ANALYSIS OF THE MUTANT STREPTOMYCES  
CLAVULIGERUS PSCL4-: ACTIVATION OF CRYPTIC BIOSYNTHETIC GENE  
CLUSTERS**

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**Background**

*S. clavuligerus* is a versatile and industrially important strain characterized by its ability to produce several economic relevant bioactive compounds such as cephamycin C and the  $\beta$ -lactamase inhibitor clavulanic acid. *S. clavuligerus* genome harbours 48 gene clusters encoding potential secondary metabolites. Under experimental conditions these clusters are not expressed ("silent clusters"), are expressed at very low levels producing undetectable amounts of the compound and in many cases the final product structure encoded by the cluster is unknown ("cryptic clusters"). A large part (21%) of the wild type genome is located in a 1.8 Mb megaplasmid that greatly influences secondary metabolites biosynthesis even if the secondary metabolites are chromosomally encoded.

**Objectives**

The objective is to analyze the differential expression between the wild strain and a pSCL4-deleted mutant and identify the activation of cryptic gene clusters in this mutant.

**Methods**

Deletion of pSCL4 was performed on the wild type *S. clavuligerus* ATCC 27064 using the Redirect Method . Microarray (*Agilent 8x15K*) data were normalized and analyzed with the *Bioconductor package limma*.

**Conclusions**

We demonstrated that the absence of linear megaplasmid pSCL4 induced holomycin overproduction, (*S. clavuligerus* is holomycin non-producer). The transcriptomic results have shown the expression of several cryptic gene clusters in *S. clavuligerus* pSCL4-. Therefore, the extrachromosomal-loss elements may be a strategy for the cryptic clusters activation in this microorganism.



**FEMS-3074**

**Global regulatory networks**

## **CHARACTERIZATION OF SFBR, AN AUTOREGULATOR RECEPTOR FROM STREPTOMYCES FILIPINENSIS**

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### **Background**

Gamma-butyrolactones are small signaling molecules that are mainly produced by *Streptomyces* species. They have been shown to affect secondary metabolism and/or morphological differentiation by binding to specific cytoplasmic receptors and therefore inhibiting their binding to target DNA sequences.

### **Objectives**

Identification and characterization of a gamma-butyrolactone receptor (GBR) encoding gene from *Streptomyces filipinensis*, the industrial producer of the polyene antibiotic filipin.

### **Methods**

A genomic library of *S. filipinensis* DSM 40112 was screened with a probe designed from a HTH DNA-binding motif conserved among autoregulator receptors. A putative receptor encoding gene (*sfbR*) was found and sequenced. Characterization of this gene was performed using gene replacement and complementation techniques.

### **Conclusions**

The *sfbR* gene encodes a putative gamma-butyrolactone receptor protein which shows homology with other described GBRs such as AvaR from *S. avermitilis* or Brp from *S. clavuligerus*. Contrary to what occurs with many GBR-encoding genes, an adjacent gene encoding a gamma-butyrolactone biosynthetic enzyme was not found. Gene deletion resulted in a significant decrease in filipin production while complementation restored parental production, thus suggesting that SfbR positively controls the filipin biosynthetic genes. Moreover, *sfbR* deletion resulted in altered morphological differentiation when the mutant strain was grown on different solid media. Our results indicate that the SfbR protein regulates both sporulation and filipin production in *S. filipinensis*.





**FEMS-1373**

**Global regulatory networks**

**PSEUDOMONAS FLUORESCENS LIPIDOME SCREENING: EFFECT OF TEMPERATURE AND GROWTH PHASE ON PSEUDOMONAS PHOSPHOLIPIDS**

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**Background**

*Pseudomonas fluorescens* spp. are present in all major natural environments, and able to quickly adapt to environmental modifications. Physiologically this adaptation depends primarily on membrane lipid composition and on its capacity to adjust membrane lipids in terms of environmental modifications. Previously we have already characterized the phospholipid composition of *P. fluorescens* [1].

**Objectives**

The aim of this work was to establish if the response to increasing temperature involves changes in the structure and the composition of *P. fluorescens* phospholipids at different phases of growth. The lipidomes of two *P. fluorescens* strains from different ecological niches : one clinical isolate MFN1032 and one airborne MFAF76a, were investigated at 28°C and 37°C.

**Methods**

To investigate *Pseudomonas* lipidome, MALDI-TOF MS Imaging was coupled to HPTLC. Additionally, NMR spectroscopy led to precise the phospholipid head group identification.

**Conclusions**

In stationary growth phase *P. fluorescens* produces a new phospholipid class: phosphoaminolipid (PAL).

Results provide evidence that next to the increasing of the temperature *P. fluorescens* is able to modify its phospholipid composition using two pathways. First the increasing of degree of unsaturation of fatty acid chains controls the membrane fluidity. Additionally the increase of the synthesis of anionic phospholipids (e.g. phosphatidylglycerol) results in the membrane charge changes. Physiologically, these two pathways allow *P. fluorescens* to adapt to stress conditions.

## References

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**FEMS-1593**

**Global regulatory networks**

**FINE TUNING OF METABOLISM: THE ROLE OF CRC/CRCZ-CRCY SYSTEM IN INTEGRATING GLUCONEOGENIC AND GLYCOLYTIC METABOLISM IN PSEUDOMONAS PUTIDA**

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**Background**

In *P. putida*, the Crc protein and the small RNAs CrcZ and CrcY are key players in carbon catabolite repression (CCR), which facilitates the assimilation of the most efficient carbon sources, improving growth rate and fitness. Succinate and glucose are good carbon sources for *P. putida*, but elicit a weak CCR. Little information is available on the regulatory and metabolic interactions when both compounds are present. The choice between them is crucial on driving a metabolism focused on glucose consumption (glycolytic), or an inverse configuration directed to gluconeogenesis (succinate consumption and synthesis of sugars).

**Objectives**

The aim was to clarify whether there is a preference for succinate or glucose, and whether the Crc-dependent CCR has a role in coordinating metabolism when cells grow with a mixture of succinate and glucose at non-limiting concentrations.

**Methods**

We undertook an integrative approach combining metabolic, transcriptomics and constraints-based metabolic flux analyses.

**Conclusions**

Succinate was consumed faster than glucose and allowed a higher growth rate. However, both compounds were co-metabolised when provided simultaneously. CrcZ and CrcY levels were lower when both substrates were present than when only one of them was provided, suggesting a role for Crc in coordinating metabolism. Flux distribution analyses suggested that, when both substrates are present, Crc works to organize a metabolism in which carbon compounds flow in two opposite directions: from glucose to pyruvate, and from succinate to pyruvate. Therefore, Crc serves not only to favour the assimilation of preferred compounds, but also to balance the carbon fluxes, optimising metabolism and growth.

**FEMS-1852**

## **Global regulatory networks**

### **SEVERAL ALTERATIONS IN PHYSIOLOGY OF YERSINIA ENTEROCOLITICA O:3 HFQ MUTANT ARE MEDIATED BY OVEREXPRESSION OF LRHA.**

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#### **Background**

In previous studies we observed that Hfq strongly downregulates the LrhA (LysR homologue A) expression in *Yersinia enterocolitica*. Hfq was first identified as a host bacterial factor required for the replication of bacteriophage Q $\beta$  RNA. Now it is known that Hfq plays a crucial role in global post-transcriptional regulation network as an RNA chaperone that binds and stabilizes small regulatory RNAs. On the other hand, LrhA was shown to regulate negatively motility, chemotaxis and flagella synthesis in *Escherichia coli*.

#### **Objectives**

In this work we wanted to elucidate the role that LrhA plays in the phenotype of the *hfq* mutant of *Y. enterocolitica*.

#### **Methods**

The *hfq* and *lrhA* genes were knocked out to create single and double mutants for subsequent analyses. The mutant strains were complemented *in trans* by functional *hfq* and *lrhA* genes. LrhA was overexpressed from an IPTG-inducible plasmid. The strains were analysed for motility, biofilm formation, growth rate and mannitol utilization affected in the *hfq* single mutant.

#### **Conclusions**

In the *lrhA hfq* double mutant we could observe partial or full restoration of such features as growth rate, motility, biofilm production and mannitol utilization. On the other hand, overexpression of LrhA in wild type bacteria caused hypermotility and different colony morphology on mannitol-containing *Yersinia* selective agar plates. Our results thus showed that several alterations in physiology present in the *hfq* mutant of *Y. enterocolitica* are mediated by increased expression of LrhA.

**FEMS-1980**

**Global regulatory networks**

**AZOARCUS ANAEROBIUS 1,3-DIHYDROXYBENZENE (RESORCINOL)  
ANAEROBIC DEGRADATION PATHWAY IS CONTROLLED BY THE  
COORDINATED ACTIVITY OF TWO SIGMA-54-DEPENDENT REGULATORS**

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**Background**

The obligate anaerobe *Azoarcus anaerobius* degrades resorcinol by an oxidative reaction where the aromatic ring is hydroxylated at position 4 to render hydroxyhydroquinone (HHQ)<sup>1</sup>. The three enzymatic steps are transcribed in three different operons controlled by two bacterial enhancer-binding proteins (bEBP) that share 97% identity, RedR1 and RedR2<sup>2,3</sup>.

**Objectives**

The work aims at understanding the coordinate expression of the pathway genes in response to the presence of the substrate by elucidating the specific activation mechanism of each regulator.

**Methods**

Genetic and biochemical approaches were used to define the activation mechanism of the two regulators.

**Conclusions**

Despite the high sequence identity between RedR1 and RedR2, they are controlled by different activation mechanisms: in the absence of effector, RedR2 is kept inactive through binding to the membrane protein BtdS, the small subunit of HHQ dehydrogenase. The presence of the substrate favors electron flow through BtdS, allowing unbinding of RedR2 in an active conformation to induce low expression levels of the enzymes. RedR1 then becomes activated by the pathway intermediate hydroxybenzoquinone. Both regulators are required for maximum pathway expression, suggesting an amplification regulatory cascade is required for coordinate expression of the entire pathway. The structure of the pathway promoters and

regulator binding have been characterized.

#### References

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**FEMS-0674**

**Global regulatory networks**

## **REGULATION OF CSRA EXPRESSION BY TWO COMPONENT SYSTEMS IN E. COLI**

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### **Background**

Global regulators play major roles in response to a variety of intracellular and extracellular signals, allowing bacteria to adapt to environmental changes.

We are particularly interested in CsrA, a post-transcriptional global regulator acting by binding target mRNAs and thereby modulating translation either negatively or positively.

CsrA is important for cell growth and regulates central carbon metabolism and social behaviors.

Transcriptional regulation of *csrA* expression remains largely unknown although it was shown that Sigma70 and RpoS directly regulate *csrA* expression. At the post-translational level, activity of CsrA is regulated by 2 major sRNAs, *csrB* and *csrC*, which bind CsrA and sequester it away from its targets. Expression of *csrB* and *csrC* is regulated by the BarA/UvrY two-component system.

### **Objectives**

Since CsrA controls pathways that are influenced by environmental conditions, it is likely that *csrA* expression is regulated by two-component systems.

### **Methods**

A screen using *lacZ* transcriptional fusions with the *csrA* promoter was set up to test two-component system mutants. In addition, bioinformatics analysis was performed to determine response regulator binding sites in the *csrA* promoter.

### **Conclusions**



Our results indicate that *csrA* expression is positively regulated by the Cpx two-component system. The Cpx system responds to a broad range of stimuli including pH, salt, lipids and misfolded proteins that cause perturbation in the envelope.

Bioinformatics analysis predicted potential CpxR binding sites in the *csrA* promoter region. EMSA and mutagenesis experiments will be performed to whether Cpx directly regulates *csrA* expression.

## FEMS-0765

### Global regulatory networks

#### CHARACTERIZATION OF THE ESCHERICHIA COLI SIGMA-S CORE REGULON BY CHROMATIN IMMUNOPRECIPITATION-SEQUENCING (CHIP-SEQ) ANALYSIS.

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#### Background

In bacteria, selective promoter recognition by RNA polymerase is achieved by its association with  $\sigma$  factors, accessory subunits able to direct RNA polymerase “core enzyme” (E) to different promoter sequences.

#### Objectives

The aim of this work was to identify promoters bound by  $E\sigma^S$ , an RNA polymerase form mainly active during slow growth and in response to stress conditions, by using a Chromatin Immunoprecipitation-sequencing (ChIP-seq) approach.

#### Methods

ChIP-seq experiments were performed in a *Escherichia coli* strain carrying a chromosomal  $rpoS_{His6}$  allele grown to stationary phase in rich medium.

#### Conclusions

We identified 78  $E\sigma^S$  binding sites, 63 of which (81%) were located in intergenic regions, mostly overlapping known or putative promoters. The majority of the  $E\sigma^S$ -bound intergenic regions was located upstream of genes (encoding either ORFs or non-coding RNAs) showing some degree of dependence on the  $\sigma^S$ -encoding  $rpoS$  gene. The remaining  $E\sigma^S$ -bound intergenic regions overlapped promoters recognized by RNA polymerase associated with the housekeeping  $\sigma^{70}$  factor, or even with the alternative  $\sigma^E$  and  $\sigma^H$  factors. Transcription of this latter set of genes does not appear to be affected by  $rpoS$  inactivation. Thus, consistent with previous observations, our

results indicate significant overlap in promoter recognition between  $\sigma^S$  and other  $\sigma$  factors.

In particular, our results suggest that, in conditions leading to  $\sigma^S$  accumulation,  $E\sigma^S$  significantly contributes to transcription of genes involved in LPS biosynthesis not belonging to the *rpoS* regulon during transition from exponential to stationary phase.

Finally, our results highlight a direct role of  $E\sigma^S$  in the regulation of non-coding RNAs

**FEMS-1991**

**Global regulatory networks**

**CHIP-SEQ AND RNA-SEQ DATA INTEGRATION TO SYSTEMATICALLY  
(RE)DEFINE THE ROLE OF NIKR IN THE HELICOBACTER PYLORI  
TRANSCRIPTIONAL REGULATORY NETWORK**

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**Background**

*Helicobacter pylori* is a human pathogen surviving in the prohibitive gastric environment where it can cause cancer. The ability to counteract environmental stresses and host responses is a key feature for persistence and depends on the rapid and fine-tuned expression of the genetic repertoire orchestrated by a shallow Transcriptional Regulatory Network. Metal-dependent regulators NikR and Fur are key hubs in this network; they directly control metal ion homeostasis and indirectly impact on several processes involved in *H. pylori* virulence and survival.

**Objectives**

In this work we combined ChIP- and RNA-sequencing to gain a comprehensive view of the NikR regulome. Moreover we investigated NikR regulation in presence or absence of Ni<sup>2+</sup> in wild-type and in *nikR* or *fur* knock-out strains, aiming to elucidate the plasticity of the system and the level of cooperation and antagonism between these two transcription factors.

**Methods**

For each experimental condition we determined NikR binding regions and defined the link between each binding site and its putative gene/operon target. Then we performed a differential peak calling to classify basal, Ni<sup>2+</sup> driven and Fur coordinated bindings. In parallel differentially expressed genes, operons and ncRNAs were identified. Finally, we discriminated direct and indirect effects of NikR action by integrating ChIP-seq and RNA-seq results.

**Conclusions**

Our study confirms known targets identified through single-gene analysis and sheds light on the entire framework of dynamic interactions between NikR and its regulatory targets, unraveling new explanations for its pivotal function in the persistence and pathogenicity of the bacterium.

**FEMS-2051**

**Global regulatory networks**

**COMPLEMENTATION AND PHENOTYPIC CHARACTERIZATION OF MUTANTS FOR NUCLEOID ASSOCIATE PROTEINS (NAPS) IN SALMONELLA ENTERICA SEROVAR ENTERITIDIS**

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**Background**

*Salmonella* Enteritidis is the most prevalent serotype in foodborne outbreaks since 1990 associated to poultry meat, eggs and products. The phagotype 4 is among most commonly isolates associated with diarrhea and systemic infection. Due to importance of this pathogen in human and animal health, researchers are searching ways to control *S. enterica* infections using vaccines. Despite the efforts of many groups, a few live vaccine strains are released for use in the salmonellosis control and there are no human vaccines to serovar Enteritidis yet.

**Objectives**

Based on these data, and taking account that motility is an important virulence trait to *Salmonella enterica*, the goal of this study was evaluate the motility phenotype of *S. Enteritidis* PT4 null mutants to genes coding to Naps and their complemented strains in comparison to wild-type strain (WT).

**Methods**

The single mutants to NAPs were constructed by  $\lambda$  Red methodology and the complementation was performed using the cloning vector pACYC184. The motility was determined by measurement of bacteria migration from the center to the periphery of Luria Bertani 0.35% agar plates.

**Conclusions**

The data were analyzed by test-T using the software *Assistat* version 7.7. The *S. Enteritidis* PT4 mutants showed a significant decrease in motility (20-55%) compared to wild-type strain ( $p < 0.01$ ). The complemented mutants reversed the phenotype, presenting motility similar to WT. Thus, we concluded that deletion of these genes affects motility, suggesting that they act directly or indirectly in the regulation of motility-related genes.

**FEMS-1435**

**Global regulatory networks**

**DECIPHERING REGULATORY NETWORKS OF THE SALMONID FISH  
PATHOGEN FLAVOBACTERIUM PSYCHROPHILUM BY PRIMARY  
TRANSCRIPTOME MAPPING**

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**Background**

In the context of the fast development of aquaculture, infections by *Flavobacterium psychrophilum* are a major sanitary concern for the salmonid farming industry worldwide. Juvenile salmonids are particularly susceptible to this Gram-negative aerobic bacterium, which is able to survive outside the fish during long periods of starvation in freshwater environments.

**Objectives**

Transcriptional regulatory networks are crucial for bacteria to maintain their integrity and to adapt to changing growth conditions. Alternative sigma factors confer promoter selectivity by binding to the bacterial RNA polymerase and allow switching between various adaptative responses. How *F. psychrophilum* adapts to environmental conditions has been investigated at the molecular level by analysis of its primary transcriptome and *in silico* prediction of regulons controlled by various sigma factors.

**Methods**

The transcriptional landscape and the transcription start sites (TSS) of the virulent strain THCO2/90 isolated from a Coho salmon, have been established using RNAseq technology by analysing a pool of bacterial RNAs extracted from cells undergoing various growing conditions.

**Conclusions**

Expressed genes as well as putative expression signals and regulatory elements were listed (*i.e.* TSS, 5'-untranslated regions, non-coding and antisense RNAs). *In silico* searches for consensus promoter sequences upstream of identified TSSs revealed more than a thousand sigma-70 dependent promoters as well as three other promoters groups responding likely to alternative sigma factors. Building this detailed transcriptional map represents the first attempt to characterize regulatory networks of *F. psychrophilum*, an important pathogen of the *Bacteroidetes* phylum.

**FEMS-2887**

**Global regulatory networks**

## **PROTEOMIC AND TRANSCRIPTIONAL ANALYSES OF STRESS-RELATED GENES IN BURKHOLDERIA XENOVORANS LB400 DURING OXIDATIVE STRESS**

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### **Background**

*Burkholderia xenovorans* LB400 is a model aromatic compounds-degrading bacterium. Aerobic metabolism of aromatic compounds may produce oxidative stress in bacteria. However, the molecular mechanisms involved are not well known.

### **Objectives**

The aims of this study were to characterize bacterial stress response in *B. xenovorans* LB400 during oxidative stress and aromatic metabolism.

### **Methods**

Stress-related genes were predicted using bioinformatic tools and functional assays were performed by proteomics using high-resolution mass spectrometry and by transcriptomics using qRT-PCR. Oxidizing conditions included exposure of LB400 cells to paraquat and hydrogen peroxide. In addition, transcriptional analysis of stress-related genes from strain LB400 grown on aromatic compounds was performed.

### **Conclusions**

Paraquat induced ferritin DPS-family DNA binding protein (DpsA), alkyl hydroperoxide reductase, fumarase C, catalase, glutathion peroxidase, oxygen peroxide resistance protein (Ohr) proteins. In addition, induction of diverse reducing enzymatic systems such as ferritins and flavodoxins was observed. During hydrogen peroxide exposure, two alkyl hydroperoxide subunits and DpsA protein were induced. A transcriptional analysis indicated that during paraquat and hydrogen peroxide exposure, an up-regulation of *ahpC*, *katE*, *fumC* and *oxyR* genes was observed. During growth of strain LB400 on 3-hydroxyphenylacetate, the expression of *ahpC*, *sod*, *acn*, *fumC* and *oxyR* genes was up-regulated. The expression of *ahpC*, *sod*, and *oxyR* genes were up-regulated during growth on 4-hydroxyphenylacetate. In conclusion, this study illustrated diverse oxidative stress proteins from *B. xenovorans* LB400 that are active during oxidative stress and aromatic compounds degradation.

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**FEMS-1741**

## **Global regulatory networks**

### **EXPERIMENTAL EVOLUTION WITH A GLOBAL REGULATOR MUTANT IN ESCHERICHIA COLI**

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### **Background**

The CsrA regulatory protein is the main actor of the carbon storage regulator (Csr) network, a well- conserved regulatory network in bacteria. It acts at the post-transcriptional level by binding specific sequences on target mRNAs, thereby modulating gene expression. This global regulator controls several physiological processes, including central carbon metabolism and social behavior. Insights into CsrA roles were mostly obtained with an *E. coli* K-12 *csrA* mutant, encoding a truncated form of CsrA that retains partial activity.

### **Objectives**

This work aims at further characterizing the roles of CsrA by analyzing phenotypes presented by an *E. coli* strain deleted for the *csrA* gene.

### **Methods**

The *csrA* gene was deleted by homologous recombination in the uropathogenic *E. coli* CFT073 strain. Experimental evolution was performed. Three independent evolved mutants were extensively analyzed by combining molecular techniques, microscopy and global approaches, including comparative proteomics and whole genome sequencing.

### **Conclusions**

Deletion of *csrA* leads to severe growth defects and altered cell morphology, indicating that this gene, although not essential, is important for growth. Evolved mutants were rapidly selected (2-3 days of culture in LB medium), showing that deleting the *csrA* gene constitutes a strong selective pressure. Our data confirms that

*csrA* deletion strongly affects central metabolism and energy status. In addition, our data show that specific stress responses are constitutively induced in the deletion mutant, including phosphosugar, oxidative and envelope stress responses. This highlights the interconnection of multiple regulatory networks and their flexibility to compensate for genetic perturbations in *E. coli*.

## FEMS-1985

### Global regulatory networks

#### TWO ISOFORMS OF YJJM TRANSCRIPTION FACTOR DIFFERING IN THE DNA-BINDING DOMAIN.

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#### Background

Ashwell and Entner-Doudoroff pathways provide an alternative to glycolysis being important for bacterial colonization and motility. There are several specific proteins (UxuR, ExuR, UidR and cAMP-CRP) sensing metabolic status of the cell and switching gene expression to the optimal pathway. YjjM was also predicted to be involved in this network.

#### Objectives

The goal of this study was to investigate *yjjM* transcription and modes of regulation.

#### Methods

Alignments and structural predictions were made using BLAST, TCOFFEE and Phyre 2. Promoters were mapped with PlatProm software, single round and 5'-RACE. Expression level was measured by beta-galactosidase assays and qRT-PCR. Proteins specifically bound to the promoter DNA were detected using pull-down assays and mass-spectrometry. YjjM products were identified by Western-blot.

#### Conclusions

YjjM structure is assumed to be very similar to other regulators of the GntR family, with oligomerization/effector binding C-terminal and DNA-binding N-terminal domain. Computational and experimental data indicated that *yjjM* may have two ORFs resulting in synthesis of two proteins variable in their DNA-binding domain by 36 aminoacids. As a basis for this heterogeneity, four possible promoters were mapped nearby the *yjjM* 5'-end, with two of them located within the main ORF. Gene expression analysis, Western-blotting and pull-down assays revealed that regulatory switch from one protein product to another may occur due to the complex interference between RNA-polymerase alpha subunits and transcription factors operating in the *yjjM* regulatory region. Thus in changing growth conditions, different promoters may tune-up DNA-binding properties of YjjM by switching the ratio of its isoforms.

**FEMS-2481**

**Global regulatory networks**

**THE INTEGRATED RESPONSE OF GENOME ORGANIZATION AND ACTIVITY TO ENVIRONMENTAL CUES**

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**Background**

Loop formation is key to the global organization of genomes in organisms from all three domains of life. Genomes are organized dynamically and their re-modelling is implied in translating external signals into specific gene products. The bacterial chromatin protein H-NS is capable of forming bridges between DNA segments *in vitro* and thus a prime candidate for genomic loop formation *in vivo*. H-NS is also a global regulator of transcription.

**Objectives**

We have investigated how the DNA bridging activity of H-NS is fine-tuned by salt conditions and interaction with co-regulators Hha and YdgT.

**Methods**

We demonstrate, using a combination of biophysical (Tethered Particle Motion), biochemical (*in vitro* bridging assays) and computational (Molecular Dynamics simulations) techniques, that there is a delicate balance between two DNA binding modes ( DNA stiffening and bridging), which can be shifted by environmental cues or H-NS interaction partners.

**Conclusions**

Our observations yield models for fine-tuning of genome organization and for the translation of changes in environmental conditions into transcriptional activity.

## **FEMS-2802**

### **Global regulatory networks**

#### **MALR - THE POSITIVE REGULATOR OF THE MAL OPERON IN THE CRENARCHAEON SULFOLOBUS ACIDOCALDARIUS**

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**Background** The thermoacidophilic crenarchaeon *Sulfolobus acidocaldarius* can utilize different carbon sources including maltose. In Archaea, sugar uptake is achieved by ABC transporters that are inducible by the corresponding sugars. How these transporters are regulated in Sulfolobales is an open question, since no regulatory proteins have been identified for any sugar ABC transporter so far. The regulation of the maltose uptake and utilization system is of special interest, since this system lies at the basis of the successful inducible expression system in *S. acidocaldarius*.

**Objectives** The aim of this study is to identify and characterize the regulator of the maltose gene cluster in *S. acidocaldarius* and use this knowledge to improve the *S. acidocaldarius* protein expression system

**Methods** The maltose regulator (MalR) of *S. acidocaldarius* was identified based on its sequence similarity to proteins of the TrmB family of regulators. To confirm the role of MalR growth of a deletion mutant was monitored on different carbon sources. Quantitative RT-PCR was used to examine transcription levels of the maltose gene cluster in a maltose-induced or uninduced state. Electromobility shift assays showed that MalR binds the promoter of the maltose binding protein (malE). Improvement of the current protein expression system was achieved by cloning an additional copy of malR into the expression plasmid.

**Conclusion** The maltose regulator of *Sulfolobus acidocaldarius* was identified. It positively controls transcription of the mal operon harboring genes for maltose uptake and metabolism. With the gained knowledge about the maltose regulator we could improve the maltose inducible expression system in *S. acidocaldarius*.

## FEMS-2100

### High-throughput approaches

#### RAPID BACILLUS PUMILUS GROUP SPECIES DIFFERENTIATION USING MALDI-TOF-MS AND FTIR-ATR

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#### Background

*Bacillus pumilus* group members, which encompass the closely related *Bacillus pumilus*, *Bacillus safensis*, *Bacillus altitudinis*, *Bacillus xiamenensis* and the newly described *Bacillus invictae*, possess a high medical, biotechnological and industrial relevance, stressing the need for reliable, easy and rapid methodologies for their correct identification.

#### Objectives

In this study we demonstrated the suitability of Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS) and Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) to accurately and rapidly identify the five closely related species enclosed within *B. pumilus* group, which are difficult to differentiate by conventional and rDNA 16S sequencing methodologies.

#### Methods

MALDI-TOF-MS and FTIR-ATR were applied to discriminate a clonally diverse collection comprising *B. pumilus* (n=5), *B. safensis* (n=19), *B. altitudinis* (n=1), *B. xiamenensis* (n=1) and *B. invictae* (n=5). Snapshots of different protein composition were acquired with a MALDI-TOF/TOF mass spectrometer, operating in linear positive mode, within the ion range at m/z 2000–12000 and using  $\alpha$ -cyano-4-hydroxycinnamic-acid as a matrix. Moreover, the whole-cell content spectra were acquired using a FTIR-ATR System with a PIKE accessory, between 4000 and 400 cm<sup>-1</sup>, with a resolution of 4 cm<sup>-1</sup> and 32 scan co-additions. Both, mass and infrared spectra were analysed by multivariate data analysis.

#### Conclusions

The resulting unique protein profile and the differences gathered in the carbohydrates and phospholipids/DNA/RNA vibration regions obtained by these high throughput approaches, proved that MALDI-TOF-MS and FTIR-ATR are valuable tools for *B. pumilus* group species discrimination, and thus, should be promptly considered for

these species identification.

**FEMS-1273**

**High-throughput approaches**

**ASSESSING THE IMPACT OF TRANSGENIC GRAPEVINE ROOTSTOCKS ON THE ENVIRONMENT USING NGS**

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**Background**

*Grapevine fanleaf virus* (GFLV) is responsible for fanleaf degeneration, which is the most severe virus disease of grapevine worldwide. GFLV causes important economic losses by reducing grape yield, lowering fruit quality, and shortening the longevity of vines. Moreover, with the ban of nematicides and soil disinfectants, fallows over 8 years are the only mean to get rid of the disease in the soil. Amongst the new strategies developed to control fanleaf, genetic engineering to modify the rootstock relying on the concept of pathogen-derived resistance, is the most promising strategy to develop GFLV-resistant grapevines.

**Objectives**

These impact studies aimed to evaluate the potential environmental consequences of the presence of two transgenes (GFLV coat protein gene and *nptII* kanamycin resistance gene) expressed in GM rootstocks, the scion remaining wild-type.

**Methods**

This study was performed in the frame of a French project (ANR IMA-GMO), that will take advantage of next generation sequencing (454 and Illumina MiSeq) technologies and quantitative PCR, to analyze the potential impact of GM plant growth either via expression of transgenes or via possible transgene transfer events (horizontal gene transfer) to microbial populations from a reference soil (Rothamsted Appletree, UK) or a vineyard soil (Bergheim, F), irrespective of the cultivability of these microbes.

**Conclusions**

Preliminary results obtained from confined experiments show no significant major modifications in the structure of the bacterial populations, and no horizontal gene transfer. Our results further stressed the necessity to perform environmental impact evaluation in long-term experiments under real agronomical conditions, in open-field trials.



**FEMS-2153**

**High-throughput approaches**

## **SCREENING FOR SMALL MOLECULE INHIBITORS OF SALMONELLA TYPE III SECRETION**

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### **Background**

Bacterial resistance to currently available antibiotics is becoming increasingly problematic. In the search for novel antimicrobial substances our strategy is to screen for substances which block essential bacterial pathogenicity mechanisms without killing the bacteria.

*Salmonella* is a major cause of food-borne diseases. It relies on two type III secretion systems (T3SS) for host cell invasion and intracellular survival inside the host cells. Selective disruption of these systems would block host cell invasion and leave the bacteria vulnerable to the host immune system.

T3SS are highly conserved across a broad range of Gram-negative bacteria. It is tempting to expect that compounds active against *Salmonella* T3SS will also affect other species such as *Yersinia*, *Shigella* and *Escherichia*.

### **Objectives**

The aim of this project is to develop a screening assay compatible with high-throughput screening and use this assay to screen for anti-virulence compounds against *Salmonella* T3SS.

### **Methods**

We adapted a standard secretion assay to 386-well ELISA format. Bacteria are grown directly in the plates in presence of small molecule compounds. Secreted effectors are adsorbed to the assay plate and subsequently quantified. Various methods for detection and quantification of secreted effectors were evaluated for their sensitivity and robustness.

### **Conclusions**

We have developed a screening approach to search for anti-infective pathogenicity-blocking small molecule compounds, which do not affect bacterial viability. Using this screening assay, we screened multiple compound libraries for active compounds. Potential actives were thoroughly validated in additional assays. In depth testing of the most promising candidates is currently underway.

**FEMS-0641**

**High-throughput approaches**

**INFLUENCE OF COLD AND OSMOTIC STRESS ON TRANSCRIPTION AND TRANSLATION IN ESCHERICHIA COLI O157:H7 SAKAI**

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**Background**

The enteric pathogen *Escherichia coli* O157:H7 Sakai (EHEC) expresses different sets of genes dependent on growth conditions. Growth at environmental conditions or in food comprises other challenges than growth in the host. Commonly, transcription intensities are compared between different conditions to obtain information about specific gene regulations. Indeed, changes in transcription levels are informative and have resulted in interesting findings in the past. However, many examples of poor correlation between mRNA and protein level exist due to translational or posttranslational regulation.

**Objectives**

In the past, transcriptomes for defined sets of genes were derived by microarrays. Today, RNAseq allows investigation of complete genomes with wider dynamic range and higher sensitivity compared to the latter technique. In addition, translationally regulated genes can be identified by ribosomal footprinting, where a snap shot of ongoing translation is obtained (translatome).

**Methods**

Strand-specific transcriptomes and translatomes of EHEC were analyzed at two different conditions (control: BHI, 37°C; stress: BHI, 14°C,  $a_w$  0.976) to examine which genes show altered expression and to distinguish transcriptional from translational regulation. For translatome sequencing ribosomal movement is stopped by chloramphenicol, unprotected mRNA is digested by RNases, ribosomes are isolated, and purified mRNA is sequenced.

**Conclusions**

Differences in transcription point, as expected, towards cold and osmotic stress inducible genes. However, the additional translation profiles obtained complete the picture. Examples of translationally regulated genes are presented which do not show changes at the mRNA level, but altered numbers of ribosomal footprints. The regulation might be achieved by, e.g., RNA thermometers.

**FEMS-1740**

**High-throughput approaches**

## **HIGH-THROUGHPUT SCREENING OF LACTOBACILLUS SPECIES FOR ANTI-MICROBIAL ACTIVITY COMBINED WITH A COMPARATIVE GENOMIC APPROACH**

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### **Background**

Lactobacilli are frequently isolated from fermented food-products and are used in starter and protective cultures to control fermentation and avoid spoilage.

### **Objectives**

To identify genes involved in fermentation performance of lactobacilli using broad genotypic/phenotypic screenings with focus on antimicrobial activity.

### **Methods**

A new high-throughput screening technique was developed to rapidly detect antimicrobial activity, using an agar diffusion assay in microtiter plates. All secondary effects like low pH, organic acids or peroxide production were controlled with buffering the media and catalase treatment. Heat and protease treatment were performed to identify bacteriocins. The approach allows screening of test conditions with 10'000 datasets per day, 5'000 antibacterial interactions per day and 3'000 antifungal/antiyeast interactions per day.

We screened 500 Lactobacilli strains from 22 species mainly derived from food environments. A total of 28 conditions were tested including peroxide, bile salt and heat resistance. Further antimicrobial activity was analyzed. We found 65 bacteriocin-like producing candidates and 25 candidates displaying antifungal or antiyeast activity. The antimicrobial activity was diverse and activity against all 12 tested *Enterococcus* strains, all 3 tested *Listeria* species and against *Aspergillus*, *Candida* and *Kluyveromyces* isolates was observed. In a next step pooled DNA samples of active strains were sequenced and comparative genomics performed to identify genes that are responsible for antimicrobial activity and for other tested phenotypes.

### **Conclusions**

The high-throughput methods allow us to screen lactobacilli with minimal laboratory equipment at low costs within short time, bringing phenotypic analyses in pace with next generation sequencing.

**FEMS-0915**

**High-throughput approaches**

**UNRAVELING THE PATHWAYS OF AMYLOID TOXICITY IN A BACTERIAL  
MODEL SYSTEM: THE REPA-WH1 PRIONOID**

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**Background**

Mitochondria are recurrently found to be central in protein amyloidosis. Thus to study mitochondrial physiology in response to protein amyloidosis is a hotspot in the amyloid research field. Since this is a quite challenging task, we have experimentally approached mitochondria in the bacterial model RepA-WH1 prionoid.

We had reported that RepA-WH1, a bacterial protein conformational switch (dWH1/mWH1) functional in plasmid replication, causes an amyloid proteinopathy in *Escherichia coli* hampering cell proliferation. RepA-WH1 aggregates are vertically transmissible but not infectious (horizontally transmissible), and enable conformational templating by cross-seeding *in vitro* and *in vivo*. It is thus the first bacterial prionoid. We have recently found that DnaK, the Hsp70 chaperone in *E.coli*, modulates RepA-WH1 amyloidogenesis *in vivo*.

**Objectives**

Study the pathways of amyloid toxicity of RepA-WH1 combining genomic, proteomic and physiological approaches

**Methods**

We have analyzed through “omics” the gene expression pattern of cells expressing RepA-WH1 (*Affimetrix* microarrays) and the fraction of *E.coli* proteome co-aggregated with RepA-WH1 amyloid inclusions. We have also studied membrane integrity, oxygen consumption rates, iron and ATP levels and sensibility to oxygen reactive species (ROS) in cells expressing RepA-WH1.

**Conclusions**

Our results indicate that cell damage is produced by a ROS-dependent mechanism, most likely involving Fenton chemistry. These findings support a role for free iron in amyloid cyto-toxicity, possibly on top of membrane damage by amyloid oligomers as it has been proposed for general amyloid induced cyto-toxicity in human amyloidosis.

The results presented here underline the power of the bacterial RepA-WH1 prionoid as a synthetic minimalist model system for amyloid proteinopathies.



**FEMS-1813**

**High-throughput approaches**

## **HIGH-THROUGHPUT SEQUENCING OF BACTERIA ASSOCIATED WITH COMMERCIALY IMPORTANT PELAGIC FISH**

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### **Background**

As the global demand for fish is increasing, and many marine species are fully exploited, a sustained profit in the industry has to result from improved quality and better utilisation. In Norway, the total volume of captured pelagic fish exceeded 1.1 million metric tonnes in 2013, giving a first-hand value of 730 million USD (4.8 billion NOK), where Atlantic mackerel (*Scomber scombrus*), Atlantic herring (*Clupea harengus*), blue whiting (*Micromesistius poutassou*), and capelin (*Mallotus villosus*) were the most important species. FAO estimates that 10-25 % of all captured fish are lost post-harvest, where microorganisms are of great importance in deterioration of fish products. Currently, bacteria associated with fish are mostly identified by culture-dependent methods. New approaches are needed to understand the complexities of bacterial communities and their influence in fish products, as most bacteria are unculturable.

### **Objectives**

To describe the bacterial community composition of gills, muscle tissue, skin, intestine, and stomach content of pelagic fish. In addition, examine water samples and zooplankton from the area of catch.

### **Methods**

Samples were obtained during commercial catch of important pelagic fish. Bacterial DNA were extracted by DNeasy Blood and Tissue Kit, amplified using NEXTflex 16S Amplicon-Seq Kit, and sequenced by the Illumina platform technology, prior to data processing. Results will be discussed.

### **Conclusions**

The information gathered in this study is important for a better understanding of the microbiology of pelagic fish species. This may again support a reduction of post-harvest losses and increase the profitability of the Norwegian pelagic fish industry.





## **FEMS-2761**

### **High-throughput approaches**

#### **SYNTHETIC BIOLOGY FOR THE DESIGN OF ANTIMICROBIAL PEPTIDES**

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### **Background**

The number of multi drug resistant pathogens is constantly growing and novel antibiotic substances are desperately needed in order to at least maintain the status quo. Ribosomally synthesized antimicrobial peptides are not yet exploited for human applications despite an indisputable potential. Among those, lantibiotics, a class of posttranslationally modified and thioether-ring bearing antimicrobial peptides, show desirable properties as high but specific antimicrobial activity and excellent stability.

### **Objectives**

We follow a synthetic biology approach for the generation of novel lantibiotics employing the blueprint of natural lantibiotics. Based on their structural and functional features, natural lantibiotics are dissected into modular subunits. These subunits are then shuffled to generate thousands of novel, putative active, chimeric lantibiotics. The lantibiotic genes are subsequently produced by combinatorial DNA *de novo* synthesis. These libraries will then be screened for molecules with high antimicrobial activity.

### **Methods**

To enable for high screening rates we developed a platform based on nL-sized reaction vessels (nL-reactors) that are used for peptide production and activity-screening in a single step and at rates of  $10^5$  variants per day. Library cells are grown to microcolonies within nL-reactors along with a sensor strain. Cells secreting an active antimicrobial peptide will deactivate the sensor cells. Clearance of an nL-reactor from the sensor thus indicates the presence of a strain secreting a highly active peptide.

### **Conclusions**

10<sup>4</sup> different chimeric variants derived from the module-shuffling of ten natural lantibiotics were analyzed. Several interesting antimicrobials have been isolated, demonstrating for the first time the generation of new-to-nature lantibiotics by module-shuffling.

**FEMS-2320**

**High-throughput approaches**

**LACTOCOCCAL SURFACE PROPERTIES AND THEIR ROLE IN MICROBE-MATRIX INTERACTIONS IN DAIRY PRODUCTS**

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**Background**

Microbial surface properties are highly variable and they are considered to be important for the interactions of the bacterial cell with the product matrix. Lactic acid bacteria are industrially used for the fermentation of milk to produce, e.g., yoghurt or cheese. In fresh milk, bacteria encounter particles such as casein micelles or fat droplets, while in fermented milk products, the proteins form a semi-solid casein matrix, in which fat globules and whey pockets are present. However, the extent to which the interaction of dairy starter cultures with the various matrix components is important for the result of fermentations is largely unknown.

**Objectives**

To which extent do bacterial cell surface properties influence interactions with the matrix in a fermented dairy product? Does it impact on starter functionality and what are the underlying mechanisms?

**Methods**

ZetaSizer was used to measure cell surface charge, microbial adhesion to Hydrocarbons (MATH) method - to measure surface hydrophobicity, and Flow Cytometry - to measure cell attachments to milk proteins.

**Conclusions**

Here, we analyzed the surface properties of 55 *L. lactis* strains and found big variations in bacterial cell-charge, surface hydrophobicity and cell attachment to milk proteins. As we also have the full genome sequences of these 55 strains, genotype - phenotype matching was used to identify mechanisms involved in the described surface properties. The identified properties and mechanisms and their relevance in relation to microbe-matrix interactions will be discussed.

**FEMS-0559**

**Microbial genomics, evolution, phylogeny**

**IS THE PREVALENCE OF CYCLOSPORA CAYETANENSIS IN HIV INFECTION UNDERESTIMATED?**

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**Objectives:** Cyclosporiasis can occur as a locally-acquired infection, among travelers or in patients with HIV/AIDS. In our country, the prevalence of *Cyclospora cayetanensis* are not clear, and researches about this microorganism in HIV infected individuals are limited. The aim of this study was to determine prevalence of *Cyclospora cayetanensis* in symptomatic and asymptomatic HIV infected individuals with conventional and molecular methods.

**Methods:** One-hundred stool samples of HIV infected individuals were included in this prospective clinical study. The stool samples were investigated by modified Ziehl-Neelsen staining and Real-Time Polymerase Chain Reaction (PCR) methods to determine the existence of *Cyclospora cayetanensis*. Permission to conduct this study was obtained from the local ethics committee of Istanbul University Istanbul Medical Faculty, Turkey.

**Results:** *Cyclospora cayetanensis* was found positive by Real-Time PCR in two samples (%2), and in one sample was found positive by modified Ziehl-Neelsen staining (%1).

**Conclusions:** As cyclosporiasis is an endemic disease in our country, *Cyclospora cayetanensis* should be considered in clinical cases, especially in immunocompromised or have long-term diarrhoea patients. In terms of cyclosporiasis, laboratory request would be appropriate in not only immunocompromised patients but also have a healthy immunity individuals. Also, patients with a history of overseas trips to endemic areas, a history of recurrent or long-term diarrhoea and abdominal pain complaints might be examined for *Cyclospora cayetanensis*. The use of Real-Time PCR seems logical, faster, efficient and more sensitive method for the laboratory diagnosis of *Cyclospora cayetanensis* according to microscopic methods in our study.

**FEMS-1466**

**Microbial genomics, evolution, phylogeny**

## **EVOLUTION AND PHYLOGENY OF PHYTOPLASMA ASSOCIATED WITH LEMON WITCHES' BROOM AS A FIRST RECORD FROM EGYPT**

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### **Background**

Witches' broom disease (WB) is a serious disease of acid limes. The first report of the disease was in Oman more than 30 years ago and has since spread to the United Arab Emirates, Iran and India where the destruction of 98% of lime trees occurred and has become a major limiting factor for lime production

### **Objectives**

The application of nested-PCR assays, the use of conserved sequences and sequencing the new emerged strains allow to broadly detect the evolution and virulent strains in field.

### **Methods**

Symptoms of the Phytoplasma Associated with (WB) infection were observed on limes for the first time in Egypt. The disease was biologically characterized. Cytopathological detection referred to Diene's stain was used to differentiate the phloem tissues of leaf sections from infected trees. Transmission Electron Microscopy (TEM) indicated the presence of phytoplasmas and were detected by the specific amplification of their 16S-23S rRNA gene. Polymerase chain reaction (PCR), utilizing the universal phytoplasma-specific primers (P1/P7 followed by R16F2n/R16R2). The amplified PCR fragment of 16S rRNA gene was successfully cloned and sequenced. The nucleotide sequences were published to the GenBank with accession number "KJ948653" and named as Lemon Witches' broom-Egyptian strain (LWB-Eg strain). Phylogenetic analysis was performed to identify the LWB-Eg strain through the alignment with the corresponding phytoplasma strains available in the GenBank

### **Conclusions**

This study discovered and identified the phytoplasma strain of LWB-Eg *Candidatus Phytoplasma aurantifolia* as a natural evolved causal agent from Lime WB strain from Oman (EF186828, 16SrII) which induces severe Witches' broom epidemic disease on lemon in Egypt.



**FEMS-1875**

**Microbial genomics, evolution, phylogeny**

## **ENTEROCOCCUS FAECIUM GENOME DYNAMICS IN LONG-TERM PATIENT GUT COLONIZATION**

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### **Background**

Since the mid-1980s *Enterococcus faecium*, normally a harmless gut commensal, has emerged as an important nosocomial pathogen that has acquired multiple antibiotic resistances. Patients can be colonized with multidrug-resistant *E. faecium* during hospitalization. These bacteria can remain present in the intestinal tract for a long period of time after hospital discharge.

### **Objectives**

Our aim is to analyze the genome dynamics of multi drug-resistant *E. faecium* isolates that colonized the gut of patients over a large timespan.

### **Methods**

The genomes of 96 isolates obtained over 8 years from 5 different patients were sequenced. We generated a core genome-based phylogenetic tree with these genomes, together with 70 previously published *E. faecium* genomes of diverse origins. Furthermore, we investigated highly similar subsets of patient isolates to describe micro-evolution in closely related strains.

### **Conclusions**

Whereas some patients were colonized for almost 2 years with a clonal population of *E. faecium*, other patients were colonized by a diverse population of *E. faecium* strains. Almost all isolates could be assigned to a hospital-associated sub-population of *E. faecium*. By studying the pan-genome of the strains in this patient isolate dataset, we identified gene gain and gene loss events. We also found evidence for recombination events, and for transmission of *E. faecium* strains between patients during hospitalization. This study highlights the different mechanisms that contribute to the genomic flexibility of clinical *E. faecium* strains. This may be a crucial factor in the ability of *E. faecium* to rapidly adapt to new ecological niches.

**FEMS-0708**

**Microbial genomics, evolution, phylogeny**

**SCREENING AND VALIDATION OF SUITABLE GENE INTEGRATION  
LOCATIONS ALONG THE GENOME OF CORYNEBACTERIUM GLUTAMICUM**

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**Background**

*Corynebacterium glutamicum* is a well-known amino acid producer microorganism. L-glutamic acid and L-lysine have become bulk products inside an opened worldwide market to threonine or tryptophan. Thus, the portfolio of *Corynebacterium* has been enlarged by relevant bioproducts such as biodegradable polymers and ingredients or additives in food, feed, cosmetics and pharmaceuticals. This new compounds production has been eased by the huge knowledge accumulated along the last decades in *C. glutamicum*, as well as the introduction of the genome sequencing facilities and other omics methodologies.

**Objectives**

The release of more than twelve genome sequences of *C. glutamicum* allows the location of their genes, putative pseudogenes, transposones or pro-phage sequences. These data have permitted the selection of putative dispensable genes or genome regions. However, the recent studies on small RNA (sRNA) or the description of Actinobacteria cryptic secondary metabolite biosynthetic gene clusters have forced to review the idea of 'dispensable regions'.

**Methods**

A meticulous RNAseq study of *C. glutamicum* transcriptome combined with bioinformatic analyses have allowed the update of the genome organization and the identification of confirmed intergenic regions free of possible genetic elements (sRNA, terminators and extended operons).

**Conclusions**

Consequently, a pool of these intergenic regions suitable for the integration of heterologous or homologous genes has been defined in *C. glutamicum*. In addition, several genes that enhance the oxygen uptake, primary metabolism precursors supply or the translation activity have been used to test the best region candidates. A set of validated *C. glutamicum* intergenic regions have been defined for genetic engineering applications.



**FEMS-1565**

**Microbial genomics, evolution, phylogeny**

## **SIMILARITIES AMONG PSYCHROTOLERANT BACILLUS CEREUS SENSU LATO GROUP MEMBERS SUGGEST THEIR COMMON EVOLUTIONARY HISTORY**

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### **Background**

The *Bacillus cereus* group comprises seven bacterial species with different ecological properties and impact on human activity. Besides virulence and heat-resistance of spores, these bacteria often show psychrotolerance, being a serious problem in food processing. Until now, cold adapted bacilli were noted among isolates representing different taxa of the entire group, however precise relationships between them remain unknown.

### **Objectives**

We decided to investigate (i) the frequency of psychrotolerant *B. cereus* group members, (ii) assess their enterotoxigenicity, (iii) genetic diversity and (iv) relationships between cold adapted isolates in the context of their origin.

### **Methods**

*B. cereus* group strains (N=400) were PCR screened for the presence of the psychrotolerant form of the *cspA* gene and cultivated at 7°C to identify cold-adapted bacteria. Enterotoxigenicity was assessed by PCR technique and immunochromatographic Duopath Cereus tests. Next, 16S rRNA gene sequencing and MLST typing were applied to establish diversity and genetic relationships among tested strains. Dendrograms were constructed using NJ method in Mega 5 software and index of association was calculated using START 2 software.

### **Conclusions**

Psychrotolerant isolates were found among *B. cereus*, *B. weihenstephanensis*, *B. mycoides* and *B. thuringiensis* from range of habitats in different frequency. They form one homogenous group in opposition to polymorphic mesophilic isolates, probably due to the slower multiplication and stabilizing effect of natural selection. Similarities in sequences of chromosomal genes and diversity of plasmid-borne features suggest that all the cold-adapted strains may have common evolutionary history.

Research funded by grant DEC-2011/03/B/NZ8/02835 (National Science Centre)

**FEMS-1270**

**Microbial genomics, evolution, phylogeny**

## **GENOME COMPARISONS REVEAL THE EVOLUTION OF NITROGEN FIXATION IN BURKHOLDERIA**

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### **Background**

The genus *Burkholderia* includes various rhizobial species capable of establishing nitrogen-fixing symbioses with certain legumes. The ability to biologically fix atmospheric nitrogen is conferred by the bacterial partner and may be transferred horizontally between species. Previous work suggests a unique evolutionary origin for the nitrogen fixing abilities of South African rhizobial species of *Burkholderia*.

### **Objectives**

In this study we investigated the evolutionary history of the nitrogen fixation loci in South African *Burkholderia* bacteria with rhizobial properties by making use of genome data.

### **Methods**

The publically available genomes of 16 *Burkholderia* species were used in this study. These included 4 South African rhizobia, 8 rhizobial species from other parts of the world, as well as 4 diazotrophic members of the genus. We also determined the genomes sequences for an additional three rhizobial species of *Burkholderia* from South Africa. The nitrogen fixation locus of these bacteria were identified and characterized, after which synteny was investigated and phylogenetic histories determined.

### **Conclusions**

Genome comparisons revealed three distinct structures for the nitrogen fixation locus among the 19 bacteria examined. One of these was represented by the South Africa species, while the other two were respectively represented by the diazotrophs and the rhizobial species from other parts of the world. Phylogenetic analyses suggested distinct evolutionary origins for the three types of nitrogen fixation locus. Overall, our

data indicated that horizontal gene transfer, gene rearrangement and/or gene replacement contributed to the differences observed in the architecture of the nitrogen fixation loci within the genus *Burkholderia*.

**FEMS-1099**

**Microbial genomics, evolution, phylogeny**

## **COMPARATIVE ANALYSIS OF THE GENOMES OF THE BACTERIA PANTOEA ANANATIS AND PANTOEA AGGLOMERANS**

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### **Background**

*Pantoea ananatis* causes diverse plant diseases, including fruit rot of pineapple, leaf blight of eucalyptus, brown stalk rot of maize, center rot of onion, and sheath rot of rice. This bacterium is also known as an opportunist human pathogen. *Pantoea agglomerans* causes bacteria palea browning of rice.

### **Objectives**

In the present study, the sequenced genomes of the closely related bacteria *P. ananatis* and *P. agglomerans* were compared with emphasis on genome organization and coding capacity.

### **Methods**

Whole genome DNA sequencing of bacteria was performed using Illumina sequencing via the synthesis method on an Illumina HiSeq 2000 genome analyzer.

### **Conclusions**

The genomes of *P. ananatis* strain PA13 from diseased grains of rice, and strain HY02 from onion center rot consist of a single circular chromosome consisting of ~4.58 Mb and a circular plasmid (~0.28 Mb). The genome of *P. agglomerans* strain SW2 consists of a single circular chromosome consisting of 3.95 Mb and three circular plasmids, Pagg\_1p (0.42 Mb), Pagg\_2p (0.16 Mb), and Pagg\_3p (94 Kb). Short collinear regions, which are identical to DNA sequences in the *Escherichia coli* MG1655 chromosome, were widely dispersed along the *Pantoea* genomes. The genome information and the mutant library will allow a better understanding of how *Pantoea* causes diseases on their plant hosts and can be utilized to develop effective control methods against these important plant pathogens.



**FEMS-2138**

**Microbial genomics, evolution, phylogeny**

**ANALYSIS OF METHYLOTROPHIC METABOLISM OF PARACOCCLUS AMINOVORANS JCM 7685 (ALPHAPROTEOBACTERIA)**

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**Background**

*Paracoccus aminovorans* JCM 7685 (*Alphaproteobacteria*) is a facultative methylotroph, which utilizes a wide range of C1 compounds, including methanol, methylated amines and *N,N*-dimethylformamide. Whole genome sequencing and sequence analysis revealed that the strain contains several genetic modules presumably involved in methylotrophy. Interestingly, many of these modules are unique among bacteria of the genus *Paracoccus* spp.

**Objectives**

The identified genes, predicted to be involved in C1 metabolism, show only moderate similarity to the corresponding genes of other well-known methylotrophs. Therefore, we conducted a set of experiments to confirm their functions and to analyze their regulation.

**Methods**

The mutant strains of *P. aminovorans* were constructed with application of transposon mutagenesis and gene replacement strategy. Proteins upregulated during methylotrophic growth were identified by the comparison of protein profiles of the bacteria grown on various C1 compounds (SDS-PAGE), followed by mass spectroscopy of selected protein bands. Activities of promoters were studied under methylotrophic and non-methylotrophic conditions using  $\beta$ -galactosidase assay.

**Conclusions**

Methylotrophic metabolism of *P. aminovorans* differs highly from that of the closely related, well-studied methylotroph *P. denitrificans* (type strain of the genus *Paracoccus*). The studied strain encodes not only more enzymes enabling oxidation of wider range of C1 compounds but has also completely different central metabolism of C1 subunit assimilation (the serine cycle in *P. aminovorans* vs. the Calvin cycle in *P. denitrificans*). Almost all genes activated during methylotrophic growth are encoded within large extrachromosomal replicons (chromids pAMV1 and pAMV3). Therefore it is highly probable that these genes might have been horizontally acquired, building mosaic methylotrophy pathways.

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**FEMS-2717**

**Microbial genomics, evolution, phylogeny**

## **CHARACTERISATION OF PROPIONIBACTERIUM FREUDENREICHII THROUGH COMPARATIVE GENOMICS**

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### **Background**

*Propionibacterium freudenreichii* is an industrially important bacterium recognised for its well-documented role in ripening of Swiss-type cheeses, production of active form of vitamin B<sub>12</sub>, capability to produce considerable amounts of propionate as well as for its recently acknowledged probiotic potential. Moreover, this species is characterized by hardiness and ability to adapt to various conditions such as those encountered in dairy and cereal environments. To this date only one genome sequence of *P. freudenreichii* has been published, hindering the possibility to fully explore its potential. *P. freudenreichii* is characterized by high G+C content and regions of repeated sequences, which has been an obstacle in unraveling the genomes of this species using Next Generation Sequencing methods such as Illumina and 454.

### **Objectives**

In this project we sequenced twenty-five genomes of *Propionibacteria* associated with various environments to identify pathways contributing to adaptation and to industrially important traits of *Propionibacterium freudenreichii*.

### **Methods**

The PacBio RSII sequencing technology was used as the method of choice, since it enables genomic library preparation without PCR amplification, and produces long reads (~10kbp) allowing for complete coverage of regions with repeated sequences. Genomes were annotated and analysed with various bioinformatic tools.

### **Conclusions**

Whole genome sequencing of *P. freudenreichii* is feasible with the PacBio RSII method and the produced genomes will help to elucidate the true potential of the species.

**FEMS-2823**

**Microbial genomics, evolution, phylogeny**

**OLSG (SINAC\_1600) IS AN ORNITHINE LIPID N-METHYLTRANSFERASE FROM THE PLANCTOMYCETE SINGULISPHAERA ACIDIPHILA**

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**Background**

Ornithine-lipids (**OLs**) are prokaryote-specific phosphorus-free membrane lipids. A function for **OLs** in stress conditions and in host-bacteria interactions, either symbiotic or pathogenic, has been shown in some bacteria. A variety of hydroxylations in different structural positions and its stress resistance implications have been reported. Recently, for the first time the presence of *N*-methylated **OL** derivatives had been described in the planctomycete *Singulisphaera acidiphila*. However, the gene/enzyme responsible for the methylation in *S. acidiphila* remained elusive.

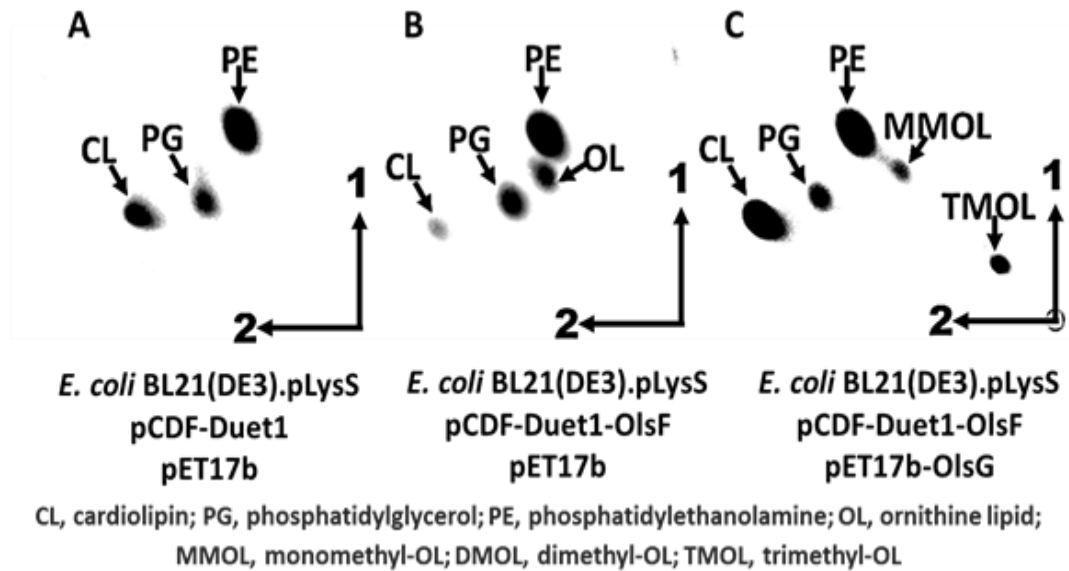
**Objectives**

Identify and characterize the novel **OL-N**-methyltransferase **OlsG** from *S. acidiphila*.

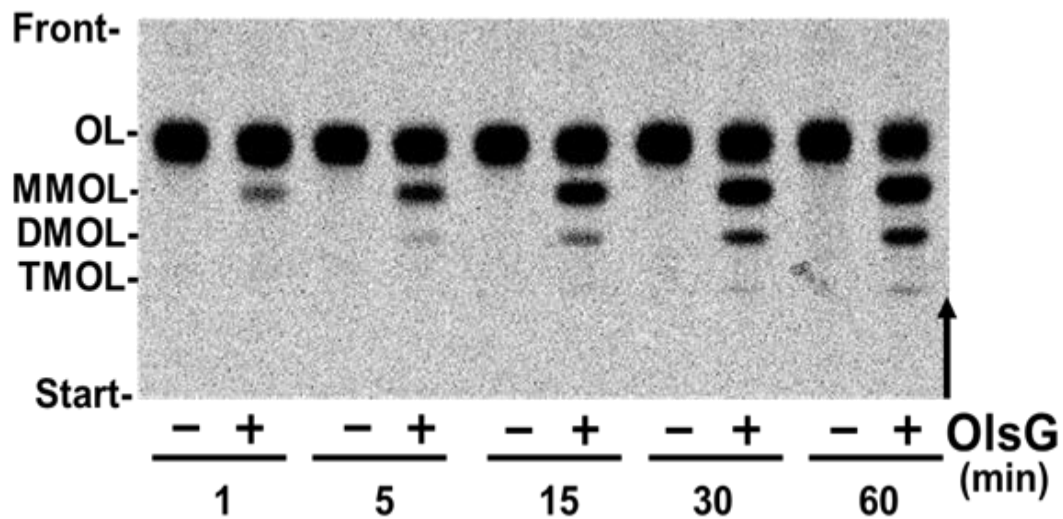
**Methods**

We identified twelve candidate genes from *S. acidiphila* encoding enzymes possibly responsible for methylated **OL** synthesis. Recently, our group identified the **OL** synthase **OlsF** from *S. proteamaculans* that upon expression in *E. coli* caused **OL** formation, so the twelve candidate genes were expressed one by one in this **OL**-forming *E. coli* strain and we analyzed the lipid composition of [<sup>14</sup>C]acetate labeled cultures of the resulting strains by two-dimensional thin-layer chromatography, leading to the identification of the novel methyltransferase **OlsG**, also confirmed by mass-spectrometry.



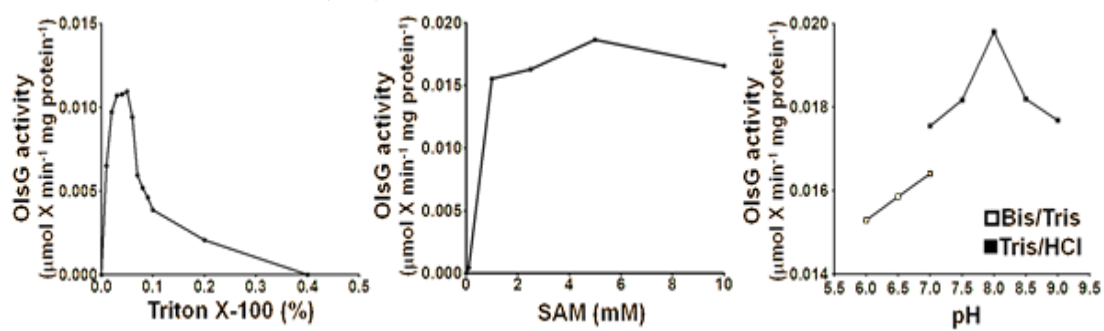


*In vitro* characterization shows that **OlsG** is responsible for the threefold **OL** methylation, resulting in monomethyl-**OL** (**MMOL**), dimethyl-**OL** (**DMOL**), and trimethyl-**OL** (**TMOL**). Time-dependency of **OL-N**-methyltransferase activity was observed.



**OlsG** contains 192 amino acids, is soluble, requires *S*-adenosylmethionine (SAM). Maximal *N*-methyltransferase activity was observed at pH=8 and in presence of

TritonX-100 [0.03-0.05%(w/v)].



## Conclusions

**OlsG** is responsible for a new type of **OL** modification.

**OlsG** is the first **OL-N**-methyltransferase described.

**FEMS-2941**

**Microbial genomics, evolution, phylogeny**

**GENOMIC ANALYSIS OF SPHINGOPYXIS (FORMERLY SPHINGOMONAS)  
MACROGOLTABIDA STRAIN TFA REVEALS NEW INSIGHTS FOR  
SPHINGOPYXIS GENUS**

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**Background**

TFA is a gram negative bacterium able to grow on tetralin as the sole carbon and energy source. Based on a partial 16S sequence it was initially ascribed to the *Sphingomonas* genus. Despite the exhaustive study of the tetralin degradation pathway, other genomic and metabolic characteristics of TFA remain unknown.

**Objectives**

The main objective is to establish the TFA metabolic map using a genomic approach and further experimental validation. New metabolic features are being experimentally validated.

**Methods**

Contigs resulting from the sequencing process were assembled. Prodigal software was used to predict protein-encoding genes, which were functionally annotated by Sma3s. The final annotation was analyzed with Pathway Tools software to generate a preliminary metabolic network. This analysis was complemented with antibiotics and secondary metabolites clusters prediction with antiSMASH. CMG-biotools, BRIG and IslandViewer were employed in comparative genomic analysis.

**Conclusions**

TFA has been re-assigned to the *Sphingopyxis* genus based on reciprocal identity comparisons versus members of the *Sphingomonadaceae* family. Highly similar regions between TFA genome and other  $\alpha$ -proteobacteria have been detected. Despite no plasmids have been detected, plasmid-related genes have been annotated, suggesting the importance of horizontal transfer in the construction of TFA genome. Comparative genomic predicts 914 TFA genes absent in other *Sphingopyxis*. A complete *nar* genes cluster encoding a membrane-bound respiratory nitrate reductase and genes for cobalamin biosynthesis seem to be exclusive characteristics of TFA.

Anaerobic growth reducing nitrate has not been described in *Sphingomonadaceae* family before. Preliminary metabolic reconstruction suggests that TFA might be able to degrade other aromatic compounds.

FEMS-2169

Microbial genomics, evolution, phylogeny

## MULTIPLE PATHWAYS FOR AEROBIC AND ANAEROBIC RESPIRATION IN MELIORIBACTER ROSEUS BELONGING TO THE NOVEL PHYLUM IGNAVIBACTERIAE

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### Background

Ignavibacteria are phylogenetically closely related to Chlorobi but essentially differ from them by their facultatively anaerobic mode of metabolism and obligate organotrophy. *Melioribacter roseus* is one of the two cultured representatives of Ignavibacteriae which grows either by fermentation of sugars and peptides, aerobic respiration or dissimilatory reduction of arsenate, nitrite or Fe(III). Genome analysis highlighted key determinants of respiratory metabolism in *M. roseus* and provided insights into its adaptation to fluctuating environmental conditions. Full set of proton-translocating membrane complexes and several putative terminal oxidoreductases were identified in the *M. roseus* genome.

### Objectives

We aimed at experimental evaluation of the role of these enzymes in energy metabolism of the organism and deciphering the mechanisms for aerobic and anaerobic respiration, basing on profound genomic analysis and experimental data.

### Methods

Inhibitor analysis and direct membrane potential measurements were used to reveal the activity of electron transfer chain in the organism. Phylogenetic analyses of the catalytic subunits of revealed oxidoreductases, transcriptomic and proteomic studies were used to confirm the function of predicted oxidoreductases in *M. roseus* metabolism.

### Conclusions

Activity of electron transfer chain under aerobic or Fe(III) respiration was revealed. Key role of a novel *cc(o/b)<sub>3</sub>*-type cytochrome oxidase and ACIII complex in aerobic respiration as well as the involvement of two non-Arr molybdopterin oxidoreductases and putative porin-cytochrome complex in anaerobic respiration were identified. Our results provide the basis for the reconstruction of the evolution of aerobic and anaerobic respiratory pathways in Chlorobi-Bacteroidetes-Ignavibacteriae group and expand the knowledge base on the novel phylum Ignavibacteriae.

**FEMS-3105**

**Microbial genomics, evolution, phylogeny**

**COMPARATIVE GENOMICS OF TOXIC AND NON-TOXIC CYANOBACTERIA STRAINS ISOLATED FROM TWO DIFFERENT BRAZILIAN FRESHWATER ECOSYSTEMS**

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**Background**

Aquatic systems are in constant transformations for the accelerated artificial eutrophication process that means nutrients enrichment. The artificial Paranoá Lake, located in Brasília (Federal District), yet in constant eutrophication process, supplies a higher degree of humidity to the local dried atmosphere. The Funil reservoir, an eutrophic environment located in Resende (Rio de Janeiro), was constructed to normalize the flow of the Paraíba do Sul River and to attenuate the impact of floods. Blooms of cyanobacteria are being reported for both aquatic ecosystems.

**Objectives**

The aim of this work is to obtain the complete genome of two cyanobacteria strains previously classified as: CYLP, non-toxic, isolated from Paranoá Lake; and CYRF, a toxic strain, isolated from Funil Reservoir; and to assess the fundamental genetic differences between the strains.

**Methods**

A next generation sequencing protocol was performed to run in Ion PGM™ platform. DNA fragments size of 400 bp from both CYLP and CYRF strains were prepared and sequencing runs were performed. Reads were downloaded from Ion Server and trimmed (quality/size) using CLC Genomics Workbench v7.5, resulting in 709Mb for CYLP and 867Mb for CYRF. The best hit species from BLASTN results was against to *Cylindrospermopsis raciborskii*. So, reads were mapped against the reference sequence CS-505 (ACYA01000000). Reads were assembled to contigs using MIRA and the final assembly was performed using CONTIGuator.

**Conclusions**

Two strains with different toxicities isolated from two Brazilian aquatic ecosystems were successfully sequenced. The assembly generated about 50 contigs. Functional analysis is being performed.

**FEMS-1880**

**Microbial genomics, evolution, phylogeny**

**GENOMIC AND FUNCTIONAL ANALYSIS OF ROMBOUTSIA ILEALIS CRIBT  
REVEALS ADAPTATION TO THE SMALL INTESTINE**

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**Background**

The microbial communities in the small intestine rely on their capacity to rapidly import and ferment available carbohydrates to survive in a complex and highly competitive ecosystem.

**Objectives**

Understanding how these communities function requires elucidating the role of its key players and the interactions among them and with their environment/host. Hence, we present the complete genome of *Romboutsia ilealis* CRIB<sup>T</sup>, a natural inhabitant and key player of the small intestine of rats.

**Methods**

The complete genome of *R. ilealis* CRIB<sup>T</sup> was sequenced and annotated, followed by a whole-genome transcriptome analysis after growth on specific carbohydrates (glucose, L-fucose and fructo-oligosaccharide [scFos]).

**Conclusions**

*R. ilealis* CRIB<sup>T</sup> possesses a circular chromosome of 2,581,778 bp and a plasmid of 6,145 bp, carrying 2351 and eight predicted protein coding sequences, respectively. Analysis of the genome reveals only limited coding capacity to synthesize amino acids and vitamins. However, multiple and partially redundant pathways for the utilization of different relatively simple carbohydrates are present in the genome. The transcriptome analysis allows pinpointing the key components in the degradation of glucose, L-fucose and fructo-oligosaccharides. This reveals that *R. ilealis* CRIB<sup>T</sup> is adapted to a nutrient-rich environment where carbohydrates, amino acids and

vitamins are abundantly available. This work shows how genome mining and functional analyses with single microbes can provide an insight in the functional potential of new members of the intestinal microbiota, and will help us with understanding the microbial communities **in us**.



**FEMS-2366**

**Microbial genomics, evolution, phylogeny**

## **MLST TO RESOLVE DISCREPANCIES IN THE IDENTIFICATION OF CRONOBACTER SPECIES**

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### **Background**

Differentiation of closely related species, such as those in the *Cronobacter* genus can be difficult. Previous characterization of *Cronobacter* isolates by phenotyping, ribotyping and 16S rRNA sequencing revealed discrepancies in the identification of certain strains (1).

### **Objectives**

This study used multilocus sequence typing (MLST) to resolve these discrepancies in the identification of *Cronobacter* species.

### **Methods**

MLST analysis was performed as previously described (2). Species identifications based on MLST were compared to previously reported phenotyping, ribotyping and 16S rRNA sequencing results (1).

### **Conclusions**

MLST confirmed ribotyping and 16S rRNA sequencing results by identifying strain 05CHPL02 as *C. dublinensis* and strain 05CHPL53 as *C. sakazakii*. These strains were misidentified biochemically. Strains 05CHPL46 and 07HMPA87A were identified as *C. malonaticus*, confirming the phenotyping and ribotyping results. Both strains were misidentified as *C. sakazakii* by 16S rRNA sequencing, likely due to the close relationship of these two species. The identification of strain 05CHPL65 as *C. sakazakii* by phenotyping and ribotyping was confirmed by MLST, though it was misidentified by 16S rRNA sequencing. Finally, strain 05CHPL47 was identified as *C. malonaticus* by MLST, whereas the other methods identified it as *C. sakazakii*.

Phenotyping, ribotyping and 16S rRNA sequencing limited in their use for the identification of *Cronobacter* spp. DNA sequence-based methods, such as MLST, are much more reliable for the identification of *Cronobacter* isolates.

## References

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**FEMS-2518**

**Microbial genomics, evolution, phylogeny**

### **IS THE RIBOSOME TARGETED BY ADAPTIVE MUTATIONS?**

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#### **Background**

RNA polymerase and ribosomes, composing the macromolecular synthesis machinery (MMSM), carry out the central processes of transcription and translation, but are usually seen as mechanical elements with no regulatory function. Extensive investigations of gene regulation and the high degree of evolutionary conservation of the cellular MMSM tend to support this view. However, under certain selective conditions the machinery itself may be targeted by adaptive mutations, which result in fitness increasing phenotypic changes. Here we investigate and characterise the role of ribosomal mutations in adaptive evolution.

#### **Objectives**

To elucidate the consequences of mutations in ribosomal protein genes in infecting bacterial isolates from human lungs.

#### **Methods**

Several mutations in ribosomal genes have been identified in the genome analysis of nearly 700 *P. aeruginosa* isolates from infected cystic fibrosis patients. Among these mutations we have repeatedly identified insertions, deletions and substitutions in specific ribosomal genes. Preliminary assays show that mutant strains have reduced growth rate and an altered antibiotic resistance pattern. The selection for mutations in ribosomal protein genes is partly explainable by the antibiotic treatment of the patient. But other mutations cannot be directly associated with antibiotic resistance. The bacterial phenotypes of the mutated strains will be investigated.

#### **Conclusions**

Clarification of the potential pleiotropic consequences of the specific mutations in ribosomal proteins is important for our understanding of biological evolution, and will have impacts on the design of new treatment strategies to combat microbial infections.



**FEMS-0803**

**Microbial genomics, evolution, phylogeny**

## **EXPRESSION ANALYSIS OF LACCASE MULTIGENE FAMILY IN TRAMETES HIRSUTA**

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### **Background**

Fungal Laccases (EC 1.10.3.2) are intensively studied due to their wide biotechnological application. However, the laccases industrial use is still limited in knowledge of enzymes biogenesis and its regulation. The regulation of laccase biosynthesis at the transcriptional level is suggested to occur via large number of specific responsive elements in the promoter regions of genes.

### **Objectives**

The work is aimed to study the regulation of multigene laccase family expression in Basidiomycete *Trametes hirsuta* – an effective lignin degrader.

### **Methods**

The full-length cDNA sequences for 7 laccases from *T.hirsuta*, using NGS techniques and RACE-PCR has been obtained. Analysis of laccases promoter regions (with MatInspector software) revealed different composition of cis-elements, including MRE, ACE, XRE, creA etc. Transcriptional dynamics for individual laccase genes were studied under application of different effectors (e.g. copper ions, different groups of xenobiotics) and confirmed with secretome analysis. The laccase complex activity was monitored during the cultivation under all conditions studied.

### **Conclusions**

The major isozyme LacA was found to be expressed in all investigated media, and apparently represented a constitutive laccase form. The expression profiles of six other laccase isozymes and laccase complex activity were different and changed depending on effectors used as well as growth phase. Analysis of transcriptional patterns and modeling of the amino acid structures of laccase isozymes allow suggesting physiological roles and functions of individual laccases, as well as biogenesis regulation within this family.

This work was supported by Grant of Russian Science Foundation № 14-14-0062

**FEMS-2828**

**Microbial genomics, evolution, phylogeny**

**INTERACTION OF BOVINE HERPESVIRUS (BHV-1) AND BACILLUS SPP.**

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**Background**

It is well known that some viruses can integrate to genome of eukaryotes. As for prokaryotes there is a special group of viruses – bacteriophages, that can interact with them. But there are almost no information about interaction of viruses pathogenic for animals and bacteria.

The aim of this work is to study possibility of integration of BHV-1 genome and bacillus.

**Methods**

Strains of bacillus were extracted from feces of cattle by standard microbiological procedure.

Screening for antigens of BHV-1 in bacilli was performed by reaction of agglutination (RA test) of bacteria with positive BHV-1 serum.

Screening for genome was done by routine and real-time PCR.

Test for induction of immunity was made by immunizing experimental group of mice with isolates of bacilli twice in 7 days interval. Control group 1 was immunized with vaccine strain of BHV-1, Control group 2 – with sterile saline. Specific antibodies were indicated in reaction of neutralization on MDBK cell culture.

For identification of bacilli automatic biotyper “Vitek” was used.

**Results**

From 70 isolates of bacilli 20 showed positive RA test. And 3 out of this 20 were positive in PCR for glycoproteins D and B genes of BHV-1.

Serums of experimental group of mice were positive for BHV-1 antibodies in dilution up to 1:40, control group 1 - up to 1:60, control group 2 - no antibodies.

Positive bacilli stains were identified as *Bacillus licheniformis*.

**Conclusions**

Obtained results show possibility of interaction and realization of genome of pathogenic viruses in bacteria in natural conditions. Also, application of this bacteria may be possible for diagnostic or prophylactic range.

**FEMS-0819**

**Microbial genomics, evolution, phylogeny**

**CONSORTIUM OF ENZYME-PRODUCING ACTINOMYCETES FROM FRUIT ORCHARD OF MUZAFFARPUR: MOLECULAR IDENTIFICATION BY 16S rRNA GENE SEQUENCE**

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**Background**

Sucrose gradient method and International Streptomyces Project (ISP) media were adopted to isolate actinomycetes in order to constitute a consortium of actinomycetes from the fruit orchard of Muzaffarpur, which were capable to produce important industrial enzymes. Microscopic, cultural and biochemical tests, followed by features-comparison as per the Bergey's Manual of Systematic Bacteriology confirmed a total of 52 strains in placing their systematic affiliation in the present investigation. The 16S rRNA gene sequencing method confirmed these species belonging to *Streptomyces*, *Nocardia*, *Nocardiopsis* and *Actinomyces*. Based on the consensus nucleotide sequences of ten strains, SK 701 to SK 710, phylogenetic dendrograms were constructed by using neighbour-joining algorithm. Two of these strains, SK 702 and SK 705 were successfully screened to have high phytase activities (380 and 315U ml<sup>-1</sup>, respectively). The optimum extracellular enzyme production was noticed at 35 °C on 4<sup>th</sup> day of culture, when the bacterium entered into late exponential or stationary phase. Though the initial pH of media was 7, however, the phytase production was stable within 3.5 to 4.5 pH.

**Objectives**

The objective is to identify the actinomycetes from fruit orchard because of their capability to produce industrial enzymes and other beneficial compounds at optimum conditions.

**Methods**

ISP and PSM were used to screen phytate-degrading actinomycetes, which were were finally phylogenetically identified by 16S rRNA gene sequencing.

**Conclusions**

Species of *Streptomyces* and *Nocardiopsis* with phytase producing ability were identified in Litchi orchard of Muzaffarpur for which PCR amplification of 643 base pairs of DNA was achieved.

**FEMS-2474**

**Microbial genomics, evolution, phylogeny**

**PHYLOGENETIC HETEROGENEITY OF TUMORIGENIC BACTERIAL STRAINS  
RECOVERED FROM RASPBERRY IN SERBIA**

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**Background**

Tumorigenic bacteria belonging to the genera *Agrobacterium* and *Rhizobium* are responsible for crown gall disease of various fruit species. During the last few years, high incidence of crown gall has been recorded in some young raspberry plantations throughout Serbia, causing considerable economic losses.

**Objectives**

The objective was to study phylogenetic structure and diversity of tumorigenic strains occurring on raspberry in Serbia.

**Methods**

A total of 14 strains were isolated from tumor tissue of diseased plants collected in six localities during 2011-2013. Tumorigenicity of strains was determined by PCR using primers specific for tumor-inducing (Ti) plasmid genes and pathogenicity assay on several test plants. PCR targeting the 23S rRNA gene, and 16S rRNA and *recA* gene sequence analysis were used to identify phylogenetic placement of strains studied. Genetic diversity of isolated strains was evaluated by using ERIC-PCR method.

**Conclusions**

Tumorigenic bacteria associated with crown gall of raspberry showed extensive genetic diversity. Out of 14 strains, 12 were identified as tumorigenic *Rhizobium rhizogenes*, one belonged to *Agrobacterium tumefaciens* genomic species G8, while the remaining strain formed separate phylogenetic lineage within *A. tumefaciens* species complex, different from all known genomic species. The fact that some geographically separated *R. rhizogenes* strains exhibited similar ERIC-PCR profiles, may indicate that they have a common origin and were probably disseminated by movement of infected plant material. This study was funded by the national III46008 and EU AREA No 316004 projects.



**FEMS-2602**

**Microbial genomics, evolution, phylogeny**

**UNDERSTANDING THE ROLE OF PII PROTEIN IN ARTHROSPIRA PLATENSIS  
PCC 7345**

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**Background**

PII proteins are known to play a crucial role in the regulation of cyanobacterial N metabolism, but its role and interacting partners in *Arthrospira (Spirulina) platensis*, a popular nutraceutical, nitrate-utilizing cyanobacterium, have not been characterized.

**Objectives**

The main aim of this study was to study the regulation of *glnB* (PII) gene by nitrate and its downstream metabolites and to screen for PII interacting proteins in *A. platensis*

**Methods**

We have cloned full length gene (*glnB*) in pET28a vector, expressed and purified PII protein by affinity chromatography for *in vitro* interaction studies. *A. platensis* cDNA yeast two hybrid library was constructed to screen for interacting partners of the PII.

**Conclusions**

Our studies have revealed transcriptional regulation of *glnB* (PII) by nitrate and its downstream metabolites. We have identified 28 colonies after library screening as potential interacting partners. Their characterization and confirmation by other approaches is underway.

**FEMS-1173**

**Microbial genomics, evolution, phylogeny**

**PHYLOGENOMIC COMPARISON OF 16 O5 SHIGATOXIGENIC ESCHERICHIA COLI (STEC) ISOLATED FROM**

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**Background**

In developed countries, *Escherichia coli* producing Shiga toxins (STEC) cause enteritis and (bloody) diarrhoea in young calves and in humans, the haemolytic–uremic syndrome (HUS) in humans, and are also present in the intestines of healthy cattle. Besides the most frequent serotypes, STEC can belong to several subdominant serotypes, like O5:H– that is present in young calves and, though more rarely, in humans and healthy cattle.

**Objectives**

The aim of this study is to analyse, at genome level, the phylogeny of 12 calf (isolated at FVM-ULg and ARSIA) and 4 human (isolated at UZ-VUB) O5 STEC belonging to different pathotypes; the *eae+stx1+* (14 isolates), *eae+stx1+stx2+* (1 isolate) or *stx1+* (1 isolate) pathotypes.

**Methods**

Genomic DNA was extracted from a growing colony and sequenced on the Illumina MiSeq platform. The raw sequences were assembled into scaffolds using an in-house pipeline and subjected to an automated annotation pipeline. The contig data were used to determine the MLST and the pathotypes. A scheme of 20 housekeeping and virulence genes were concatenated and used to select the representative strain, which will be submitted to another run of sequencing and used as the reference for a phylogenomic analysis. The phylogenomic tree will allow to analysing whether the O5 STEC strains could be clustered according to the origin parameter (human or bovine).

## **Conclusions**

Genomic sequencing has become a quick and efficient tool that can be used to replace numerous existing typing analysis methods by a unique analysis scheme.

FEMS-0967

Microbial genomics, evolution, phylogeny

## BACTERIOPHAGE-MEDIATED, HIGH EFFICIENCY HORIZONTAL GENE TRANSFER IN BACTERIAL PATHOGENS AND BIOCONTROL AGENTS

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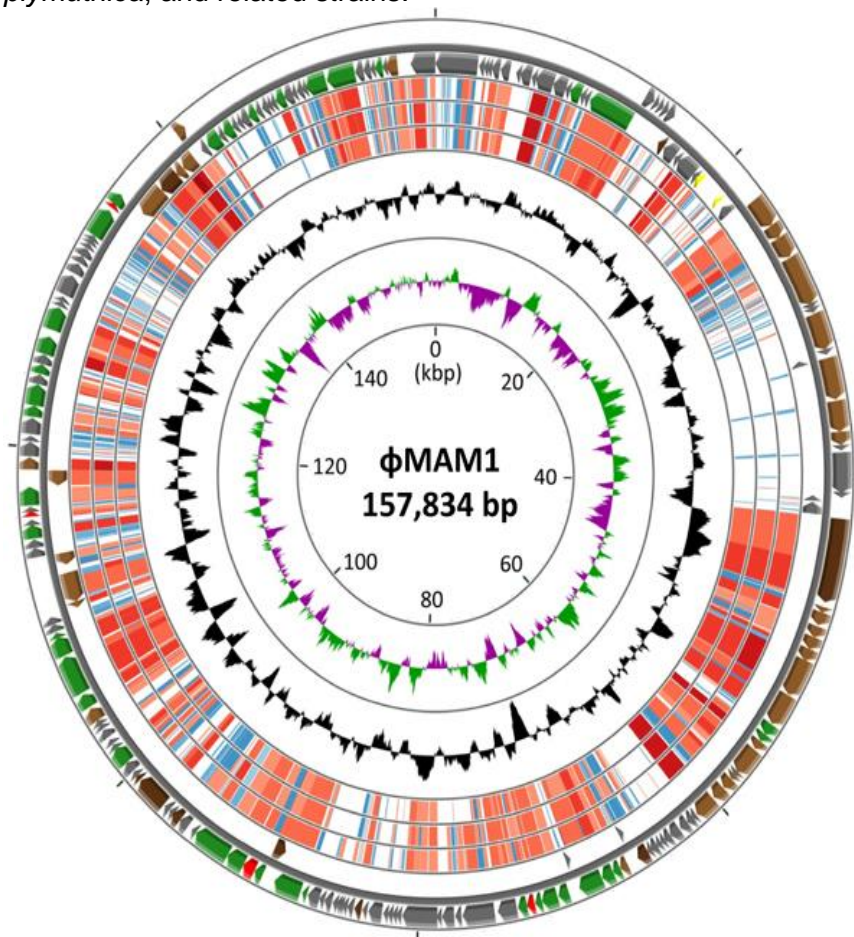
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### Background

Transduction is one of the main drivers of horizontal gene transfer in bacteria and contributes to the rapid evolution of prokaryotes. No transducing phages have yet been described for *Serratia plymuthica*, a biocontrol bacterium that produces bioactive molecules - including the antifungal macrolides, oocydins (1).

### Objectives

The aim of this work was to isolate a generalized transducing phage(s) for *Serratia plymuthica*, and related strains.



### Methods

New phages were isolated, scored for transduction capacity, phenotypically and

morphologically characterised, and genomically sequenced.

## **Conclusions**

The new *Serratia* phage,  $\phi$ MAM1 (2,3), transduced chromosomal markers at high frequencies. We demonstrated transduction of the 77-kbp oocycin gene cluster and showed heterogenic transduction of plasmids. Morphological, genomic and phylogenetic analyses grouped  $\phi$ MAM1 within the suggested new genus, Viunalikevirus (3). We show that four additional viunalikeviruses, infecting plant and human pathogens, were highly efficient generalized transducing phages. We hypothesised that related phages are likely to perform efficient horizontal gene transfer. To test this hypothesis, we first isolated several new viunalikeviruses from the environment,  $\phi$ XF1,  $\phi$ XF3 and  $\phi$ XF4. All these phages transduced chromosomal markers and plasmids at high frequencies (4). We predict that all viunalikeviruses will perform efficient horizontal gene transfer in their respective hosts. These phages will have utility for functional genomics, bacterial engineering and synthetic biology studies, but may be inappropriate candidates for use in phage therapy.

## **References:**

- 1) Matilla et al.**, (2012) *J Biol Chem* **287**:39125-38; **2) Matilla and Salmond** (2012) *J Virol* **86**:13872-3; **3) Matilla and Salmond** (2014) *Appl Environ Microbiol* **80**:6446-57; **4) Matilla et al.**, (2014) *ISME J* **8**:2143-7.

## **FEMS-2701**

### **Microbial genomics, evolution, phylogeny**

#### **THE COMPLETE GENOME OF MYCOPLASMA SYNOVIAE WVU1853**

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#### **Background**

*Mycoplasma synoviae* causes infectious synovitis, respiratory tract infections, and eggshell apex abnormalities in commercial poultry. Sequencing of the field isolate MS53 has provided a basis research exploring the virulence and evolution of this organism; however, this isolate is attenuated and is not available to laboratories worldwide. Explorations into loci of interest reveal high levels of genetic diversity between MS53 and other isolates, emphasizing the need to solve the genomes of additional strains. Past efforts have been complicated by a large repeat-rich region encoding variable surface antigens (the *vlhA* locus).

#### **Objectives**

The objective of the work was to generate a complete genome sequence for the *M. synoviae* type strain WVU1853<sup>T</sup> and compare its features to MS53.

#### **Methods**

Sequence reads were generated using a combination of Illumina MiSeq and Pacific Biosciences SMRT technologies. Genome assembly was carried out using CABOG (J. Craig Venter Institute), Newbler (454 Life Sciences), and Ray (Boisvert *et al.*, 2012) assembly software programs.

#### **Conclusions**

The *M. synoviae* WVU1853<sup>T</sup> genome was sequenced and closed. It is larger than the MS53 genome, and many notable changes in the *vlhA* locus were apparent.

**FEMS-0571**

**Microbial genomics, evolution, phylogeny**

## **NIC-GENE CLUSTER'S MODULAR DESIGN WITHIN THE ARTHROBACTER GENUS**

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### **Background**

The pAO1 megaplasmid of *Arthrobacter nicotinovor*as shows low levels of sequence similarity with ther *Arthrobacter* plasmids, but shares most of its *nic*-genes with three *Arthrobacter* draft genomes: M2012083 (GI:NZ\_AKKK000000000), SJCon (GI:NZ\_AOFD000000000) and AK-YN10 (GI: NZ\_AVPD000000000).

### **Objectives**

The **objective** of the current study is to make an evolutionary analysis of the *nic*-cluster tacking into account the arrangement and collinearity of the *nic* ORF's.

### **Methods**

The three draft genomes of the above mentioned *Arthrobacter* strains were assembled based on on the existing final *Arthrobacter* genomes using MAUVE, annotated with RAST and further aligned with Artemis. *Arthrobacter* sp. AK-YN10 (a gift from Dr. Atya Kapley, CSIR-NEERI, Nagpur, India) and pAO1 strains were grown on citrate medium supplemented with nicotine. Nicotine consumption in the medium was followed by HPLC.

### **Conclusions**

The *nic*-gene cluster can be divided into five modules, each module encoding a precise step in the nicotine-pathway. For each module, a general rule can be observed: the pAO1 modules are the most complex, with a large number of genes, including transposases and insertion elements. The SJCon modules are the most simple, with a small number of ORF's and large non-coding regions. The AK-YN10 strain is somewhere in the middle, but the five modules are spread through the genome. The test for the nicotine resistance of this bacteria has shown levels comparable with the pAO1 strain. Moreover AK-YN10 can grow on nicotine containing citrate media without forming the characteristic nicotine-blue pigment.

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**FEMS-3110**

**Microbial genomics, evolution, phylogeny**

**INSIGHTS FROM THE GENOME ANNOTATION OF THE CHROMIUM RESISTANT AND PLANT-GROWTH PROMOTING RHIZOBACTERIUM DELFTIA SP. JD2**

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**Background**

*Delftia* sp. JD2 is an auxin/siderophore plant-growth-promoting bacterium that promotes growth and nodulation of alfalfa when co-inoculated with *Sinorhizobium meliloti*. Additionally, JD2 reduces Cr(VI) to Cr(III) and shows multi-resistance to several heavy-metals and antibiotics.

**Objectives**

The aims of this work were: 1) to perform a comparative genomic analysis using the draft genome of JD2 and, 2) to analyze the molecular elements involved in JD2 phenotype, such as chromium resistance, auxin- and siderophore-production, among others.

**Methods**

We had recently sequenced the genome of JD2 and its automated annotation has been finished. We are currently analyzing the potential pathways involved in chromate resistance, auxin production, polyhydroxy-alkanoates production and accumulation, horizontal gene transfer elements and others. Results from comparative genomic analysis and different approaches to inferring phylogeny will be shown during the meeting.

**Conclusions**

JD2 has a 6.76Mb genome. The annotation and comparative genomic analysis revealed some functional characteristic associated with its previously reported plant growth promoting effects on alfalfa. JD2 has numerous genes related to tryptophan synthesis and degradation, indol 3-acetic acid biosynthesis, siderophores production and secretion and TonB dependent transporters with various substrate specificities. Consistent with its environmental adaptability, JD2 contains a large number of genes involved in the transport and efflux of toxic compounds. Syntenic genes related to

chromate resistance (*chr*) were identified. Interestingly, in adjacent positions to *chr* cluster some mobile elements (transposases and integrases) were found. The results provide insights into the potential use of JD2 in heavy metal bio-remediation programs and biofertilization technologies.

**FEMS-2692**

**Microbial genomics, evolution, phylogeny**

**SPECIES REASSIGNMENT OF ENTEROBACTER CLOACAE COMPLEX  
ISOLATES BY IN SILICO PHYLOGENETIC ANALYSIS**

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**Background**

The taxonomic resolution of closely related species belonging to *Enterobacter cloacae* complex ("EcC", *E. cloacae*, *E. hormaechei*, *E. asburiae*, *E. kobei* and *E. ludwigii*) by conventional methodologies or MALDI-TOF MS is often unreliable, and sequencing of 341bp *hsp60* has been proposed for their discrimination. Moreover, a multilocus sequence typing (MLST) assay targeting *dnaA*, *fusA*, *gyrB*, *leuS*, *pyrG*, *rplB* and *rpoB* genes has been suggested for epidemiological typing of *E. cloacae*, considered one of the most frequent species in the clinical setting.

**Objectives**

To assess by a phylogenetic approach, using different biomarkers, the distribution of EcC species in available databases.

**Methods**

A phylogenetic-based study was conducted to analyze the *hsp60* (complete gene) and individual and concatenated sequences of *dnaA*, *leuS* and *rpoB* (partial genes) from 352 *Enterobacter* sp. isolates (including type and/or reference EcC strains), deposited in the Pathosystems Resource Integration Center (Patric) and MLST databases. Phylogenetic trees were constructed using Neighbour-Joining method supported by Tamura 3-parameter model and the reliability of internal branches assessed by bootstrap from 1000 re-samplings.

**Conclusions**

The topology of the trees (individual and concatenated) congruently revealed 5 main clusters corresponding to the five known EcC species. Interestingly, *E. cloacae* were minority (3%), whereas *E. hormaechei* (63%) and *E. asburiae* (15%) were overrepresented, highlighting the current misidentification of EcC species, with potential relevant clinical implications. Finally, cut-off values of  $\leq 91.7\%$  and  $\leq 95.9\%$  are proposed, respectively, for *leuS* and *hsp60*, the most discriminatory genes for EcC species determination, which could support their reliable identification.

**FEMS-1428**

**Microbial genomics, evolution, phylogeny**

**GENETIC ANALYSIS OF THE LISTERIA PATHOGENICITY ISLAND 1 OF *L. MONOCYTOGENES* 1/2A AND 4B ISOLATES**

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**Background**

Genetic analysis of the *Listeria* Pathogenicity Island 1 (LIPI-1) as well as several other genes has been applied in order to gain insights into the evolution of *L. monocytogenes*.

**Objectives**

The aim of the present study was to apply descriptive, phylogenetic, recombination and selection analyses on alignments of the LIPI-1 of 1/2a and 4b isolates of different origin in order to facilitate understanding of the evolution of this virulence gene cluster.

**Methods**

A total of 19 *L. monocytogenes* isolates (9 meat isolates, serotype 1/2a; 5 meat isolates, serotype 4b; 5 strawberry isolates, serotype 4b) were subjected to sequencing of their LIPI-1. Alignments of the complete island as well as intra- and intergenic regions were used for descriptive, phylogenetic, recombination and selection analyses.

**Conclusions**

Descriptive analysis revealed extensive nucleotide diversity mostly in the intragenic regions. The *actA* gene of 1/2a and 4b meat isolates and the *hly* gene of the 4b strawberry isolates exhibited the higher diversity. On the contrary, limited diversity was observed in *prfA* and *plcA* genes of the 4b isolates and *mpl* gene of the 1/2a isolates. Phylogenetic analysis of the complete island resulted in two major clusters that were consistent with serotype assignment of the isolates. Moreover, within the 4b cluster, isolates were separated according to their isolation source. Selection analysis revealed that the island consisted of randomly evolving DNA with the exception of *prfA* gene of 1/2a isolates and *actA* gene of 4b meat isolates for which purifying selection was indicated. Finally, no statistically significant evidence for recombination has been observed.

**FEMS-1432**

**Microbial genomics, evolution, phylogeny**

## **EFFECT OF LEMONGRASS ESSENTIAL OIL ON LISTERIA MONOCYTOGENES GENE EXPRESSION**

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### **Background**

The transcriptomic response of *L. monocytogenes* to abiotic stresses has been the epicenter of intensive study over the last decade and the complexity of regulation of gene expression has been highlighted.

### **Objectives**

The aim of the present study was to assess the transcriptomic response of *L. monocytogenes* isolates on the exposure to lemongrass essential oil.

### **Methods**

Overnight culture of six strains previously isolated from a strawberry sample was spread over the surface of BHI agar and exposed to 5 and 10 uL of lemongrass essential oil that were applied on a Whatman paper placed on the lid. Three of them survived in the former case and one in the latter; in all cases biomass was collected and RNA was isolated. The expression of the key virulence genes *sigB*, *plcA*, *plcB*, *hlyA*, *inlA*, *inlB*, *inlC*, *inlJ*, *lmo2470* and *lmo2672*, as well as *accA*, *acpP* and *fapR* involved in fatty acid biosynthesis/metabolism and *murE* and *pbpB* involved in peptidoglycan biosynthesis was assessed by RT-qPCR.

### **Conclusions**

Downregulation of virulence genes was observed with the exception of *sigB* for which upregulation in two isolates was observed. On the contrary, significant differences between the isolates were observed regarding the expression of *accA*, *acpP*, *fapR*, *murE* and *pbpB*. The increased amount of lemongrass essential oil affected significantly the expression of *accA*, *pbpB*, *sigB*, *hlyA*, *inlA*, *inlB*, *inlJ* and *lmo2672*.

**FEMS-1205**

**Microbial genomics, evolution, phylogeny**

**AN HOMOLOG OF THE FRZ PHOSPHOENOLPYRUVATE: CARBOHYDRATE PHOSPHOTRANSFERASE SYSTEM OF EXTRAINTESTINAL PATHOGENIC ESCHERICHIA COLI IS ENCODED ON A GENOMIC ISLAND IN SPECIFIC LINEAGES OF STREPTOCOCCUS AGALACTIAE**

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**Background**

*Streptococcus agalactiae* and *Escherichia coli* have in common the capability to colonize and infect similar hosts, and to survive in the environment and in foods. In *E. coli*, the Frz system is involved in environmental sensing and regulation of the expression of adaptation and virulence genes. Thus, it is possible that *S. agalactiae* possesses a system similar to Frz to sense its environment and to adapt to environmental conditions.

**Objectives**

The presence of a system similar to Frz was searched in *S. agalactiae* strains representative of the genetic diversity of the species.

**Methods**

The genomes of 492 strains of *S. agalactiae* of human and animal origins were analyzed *in silico* and by PCR. The strains were characterized by multilocus sequence typing.

**Conclusions**

We identified an operon (named *fru<sub>2</sub>*) coding a system similar to Frz. *fru<sub>2</sub>* encodes three subunits of a PTS transporter of the fructose-mannitol family, a transcriptional activator of PTSs of the MtlR family, an allulose-6 phosphate-3-epimerase, a transaldolase and a transketolase. *fru<sub>2</sub>* is present in a genomic island. The showed that the presence of *fru<sub>2</sub>* is closely linked to the phylogeny of *S. agalactiae*. Our results indicate that *fru<sub>2</sub>* was acquired during the evolution of *S. agalactiae* before the divergence of CCs 1, 7, 10 and 283, and STs 130 and 288. As *S. agalactiae* strains of CCs 1 and 10 are frequently isolated from adults with invasive disease, we hypothesize that the *S. agalactiae fru<sub>2</sub>* system senses the environment to allow the bacterium to adapt to new conditions encountered during the infection of adults.

**FEMS-0890**

**Microbial genomics, evolution, phylogeny**

## **COEVOLUTION OF HUMAN AND HELICOBACTER PYLORI GENOMES SHAPES THE RISK OF GASTRIC DISEASE**

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### **Background**

*Helicobacter pylori* is the principal cause of gastric cancer (GC). However, *H. pylori* prevalence generally does not predict cancer incidence

### **Objectives**

To determine if the coevolution between host and pathogen influences the risk of GC in two Colombian populations: Tumaco, a coastal population with low risk of GC, and Tuquerres, a mountain population with high risk of GC. Our hypothesis postulates an association between the severity of gastric lesions and the patterns of genomic variation in matched human and *H. pylori* samples

### **Methods**

292 patients were recruited from the two populations. Histological diagnostics were based on the Sydney classification. 252 strains of *H. pylori* were included for MLST analysis. Human DNA was genotyped using the ImmunoChip to characterize the human ancestry. The Admixture model of STRUCTURE assigns proportions of ancestry to each individual sample

### **Conclusions**

*H. pylori* isolates with an ancestral African cluster predominating in a low-risk (coastal population), and a European cluster in a high-risk (mountain population). The human ancestries of biopsied individuals also vary geographically, with mostly African ancestry in the coastal region, and mostly Amerindian ancestry in the mountain region. The interaction between host and pathogen ancestries completely accounted for the difference in the severity of gastric lesions in the two regions of Colombia. *H. pylori* ancestry was relatively benign in humans of African ancestry but was deleterious in individuals with Amerindian ancestry. Thus, coevolution probably shapes disease risk, and the disruption of coevolved human and *H. pylori* genomes can explain the high incidence of gastric disease in mountain population.

**FEMS-2753**

**Microbial genomics, evolution, phylogeny**

**EXPLORING GENETIC DIVERSITY OF XANTHOMONAS ARBORICOLA PV. CORYLINA STRAINS AND DEVELOPMENT OF SPECIFIC MOLECULAR MARKERS**

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Background: *Xanthomonas arboricola* pv. *corylina* (Xac) is the causal agent of bacterial blight of hazelnut. The most efficient distribution of the pathogen to new geographical areas occurs through contaminated plant material. Therefore, fast and reliable pathogen detection using highly specific molecular diagnostic tools is essential for the production of disease-free planting material.

Objectives: The aim of this work was to study genetic structure and diversity of Xac strains and develop molecular tools for specific and rapid detection of the pathogen.

Methods: In order to study genetic diversity forty Xac strains originating from Serbia were initially analyzed by rep-PCR using BOX, ERIC and REP primers. Obtained genetic profiles from all three PCR methods were transformed into a binary matrix, combined and used for phylogenetic analysis and construction of the dendrogram. Seventeen representative strains with different rep-PCR patterns were selected for further macrorestriction analysis using *SpeI* restriction enzyme and pulsed-field gel electrophoresis (PFGE). Specific primers were designed based on specific DNA fragment obtained by ERIC-PCR.

Conclusions: The strains were differentiated into eight clonal and highly related genetic groups by rep-PCR analysis. Unique PFGE patterns were obtained for each representative strain indicating more polymorphism between the strains, compared to rep-PCR. Designed PCR set of primers enabled detection of Xac strains from different geographic origin. However, they could not distinguish Xac from closely related *Xanthomonas* spp.



**FEMS-3081**

**Microbial genomics, evolution, phylogeny**

## **EXPLORING DIVERSITY AND EVOLUTION OF MICROBIAL GENOMES USING NEXT-GENERATION SEQUENCING**

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### **Background**

Genome organization and sequence is dynamic, undergoing changes as part of evolutionary process based on genomic structure and environmental conditions under which organisms grow. The whole genome sequences (WGS) are useful information to understand evolutionary aspects of any organism. We have used Next-generation sequencing (NGS) to survey and analyse genomic changes occurring in different species and isolates of bacteria..

### **Objectives**

1. Identification and analysis of mutation hotspots in genome of *M.tuberculosis*.
2. Construction of phylogenetic trees from NGS datasets of microbes.
3. Identification of IS-elements from NGS datasets and understanding role of IS6110 in genome evolution of *M.tuberculosis*.

### **Methods**

1. We have used Shewhart Control Chart to identify genomic mutation hotspots in *M.tuberculosis*.
2. We have developed a method which uses random sampling of genome to reduce computational complexity to construct phylogenetic tree. It is able to construct trees in absence of reference genome, which is important for non-model organisms. This was used for NGS datasets of *M.tuberculosis*, *E.coli* and *V.cholerae*.
3. We have used a split-read approach to identify location of IS elements in microbial genome from NGS datasets. Distribution and copy number of IS6110 was identified from 1377 *M.tuberculosis* strains representing all major global lineages.

### **Conclusions**

Identification and analysis of hotspots enables to study regions responsible for diversification and phenotypic consequences such as, drug-resistance. Generation of phylogenetic trees directly from NGS reads enables study of evolutionary relationships among hundreds of closely related strains. Lineage specific IS6110 distributions were observed and gene truncation, possible promoter activity and IS mediated genomic rearrangements were studied in detail.

**FEMS-2998**

**Microbial genomics, evolution, phylogeny**

## **A POSSIBLE EVOLUTIONARY SCENARIO FOR THE LOSS OF OPGGH IN YERSINIA PESTIS.**

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### **Background**

The *opgGH* operon encodes the glycosyltransferases OpgH and OpgG, which synthesize osmoregulated periplasmic glucans (OPGs) and are required for motility, biofilm formation and virulence in various bacteria. OpgH also sequesters FtsZ, in order to control bacterial size as a function of nutrient availability. The role of *opgGH* in *Yersinia pseudotuberculosis* is unknown. Furthermore, *opgGH* was lost during the emergence of *Yersinia pestis* (the agent of plague) from *Y. pseudotuberculosis* (an enteropathogen). This loss is surprising, since *Y. pestis* is extremely virulent and flea-borne transmission of *Y. pestis* relies on biofilm formation.

### **Objectives**

To determine the role of *opgGH* in *Y. pseudotuberculosis* and consider the role of *opgGH*'s loss in the emergence of plague.

### **Methods**

Genetic approaches were used to generate (i) a  $\Delta$ *opgGH* *Y. pseudotuberculosis* strain, and (ii) *Y. pestis*,  $\Delta$ *opgGH* *Escherichia coli* and  $\Delta$ *opgGH* *Dickeya dadantii* strains expressing *Y. pseudotuberculosis* *opgGH*. Biochemical assays (used to detect OPG synthesis), motility and biofilm assays, microscopy (used to measure bacterial size) and virulence testing (using endive leaves, mice and fleas) were used to determine the impacts of the mutation and the *opgGH* expression in the strains of interest. Animal studies were performed in compliance with the French regulations.

### **Conclusions**

Our results suggest an evolutionary scenario in which an ancestral strain of *Yersinia* lost or did not acquire a cofactor that was important for OPG biosynthesis but kept *opgGH* to control its bacterial size in a nutrient-dependent manner. The *opgGH* gene was then lost presumably because the nutrient-dependent size-control mechanism was no longer necessary.

**FEMS-1838**

**Microbial genomics, evolution, phylogeny**

**PHYLOGENETIC ANALYSIS OF TREPONEMA PALLIDUM SUBSP. PALLIDUM, PERTENUE AND ENDEMICUM STRAINS: IDENTIFICATION OF POSITIVELY SELECTED GENES**

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**Background**

The genus *Treponema* comprises several uncultivable human pathogens including *Treponema pallidum* subsp. *pallidum* (TPA), the causative agent of syphilis, *T. p.* subsp. *pertenue* (TPE, the causative agent of yaws), and *T. p.* subsp. *endemicum* (TEN, the causative agent of bejel).

**Objectives**

The goal of this study was to identify positively selected genes in comparisons within and between TPA, TPE and TEN strains.

**Methods**

Altogether, nine whole genome sequences comprising 4 TPA, 4 TPE and 1 TEN strains were used to identify positively selected genes. Several software packages were used including Mega4, PAML and TimeZone in all possible pairwise combinations.

**Conclusions**

A set of 25 treponemal genes were identified among more than 1000 tested TPA, TPE and TEN orthologous genes. Eleven of these genes were found to be positively selected in TPA-TPE comparisons but not within TPA or TPE strains. In contrast, five genes including *bamA* and *mcp-2* were positively selected also within TPA and TPE strains. Two genes (TP0559, TP0966) appear to be positively selected within TPA but not within TPE strains suggesting their role in syphilis pathogenesis. Gene TP0304 is positively selected within TPE but not within TPA. Two genes (*tprF*, *I*) were found positively selected within TPA and TPE strains but not in comparison between TPA and TPE strains. Except for *tprC* gene where positive selection was identified between TPA and TPE comparisons, TEN orthologs resembled TPE orthologs. Altogether, positively selected genes encode for Tpr proteins, protein antigens (e.g. TP0326, BamA), putative lipoproteins and outer membrane proteins.

**FEMS-2633**

**Microbial genomics, evolution, phylogeny**

**KEY FEATURES FOR THE ADAPTION AND SURVIVAL OF LISTERIA MONOCYTOGENES IN THE FOOD PROCESSING ENVIRONMENT**

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**Background**

The saprophyte *L. monocytogenes* is well established in the environment.

**Objectives**

Persistent *L. monocytogenes* and *Listeria* spp. isolates (characterized by PFGE and MLST typing) are hypothesized to be better adapted to the food processing environment (FPE).

**Methods**

The whole genome of 15 *L. monocytogenes* and *Listeria* spp. genotypes established in the same food processing environment was sequenced applying the Illumina sequencing technique.

**Conclusions**

The *L. monocytogenes* core-genome was highly syntenic. Differences were detected in mobile elements of the accessory genome. Eight *L. monocytogenes* and two *L. innocua* strains indicated the presence of 15-90 kbp plasmids. The long-term *L. monocytogenes* persistent strains, assigned to genetic lineage I [sequence type (ST) 5] and lineage II (ST204), harbored a plasmid comparable to plm80. When comparing *L. monocytogenes* ST37 at the beginning and the end of isolation, the loss of a distinct phage region (~33 kbp) was noticeable. The  $\beta$ -lactamase encoding transposon Tn552, was detected in the plasmids of *L. monocytogenes* ST204 (n=1) and ST5 strains (n=6). The transposon Tn554, suspected for benzalkonium resistance, was found in *L. monocytogenes* ST9, one *L. innocua*, and *L. seeligeri*. The *L. monocytogenes* stress-survival islet (SSI-1) was present in ST5, ST204, and ST9.

The results indicate mutations in the *L. monocytogenes* ST5, ST37 phage and/or plasmid genome suspected for better adaption and survival in the particular FPE. Gene regions for better adaption to disinfectants and acidic stress (Tn554, SSI-1) were found in long term persistent *L. monocytogenes* strains.

**FEMS-3006**

**Microbial genomics, evolution, phylogeny**

**AN IMPROVED CLASSIFICATION OF DYP-TYPE PEROXIDASE FAMILY  
ACCELERATES THE RESEARCH OF THE FAMILY**

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**Background**

So far, the range of DyP-type peroxidase family was obscure. Therefore, the name of DyP-type peroxidase family and its superfamily tended to be confused.

**Objectives**

To solve this problem, we focus on the relationship between tertiary and primary structures of DyP-type peroxidases associated with their function. From the viewpoint, we propose the range of DyP-type peroxidase family reasonably.

**Methods**

Dye-decolorizing peroxidase from the basidiomycete *Bjerkandera adusta* Dec 1 (DyP) is a heme peroxidase. This name reflects its ability to degrade several anthraquinone dyes. The substrate specificity, the amino acid sequence, and the tertiary structure of DyP are different from those of the other heme peroxidase families. Therefore, many proteins showing the similar amino acid sequences to that of DyP are called DyP-type peroxidase which is a new family of heme peroxidase identified in 2007. Although all structures of this family show a similar structure fold, this family includes many proteins whose amino acid sequence identity to DyP is lower than 15%. Moreover, their catalytic efficiency ( $k_{\text{cat}}/K_{\text{m}}$ ) is a few orders of magnitude less than that of DyP. For the activity, some of them have been reported different from peroxidase activity (dechelataase activity).

**Conclusions**

Here, we report the differences and similarities of structure and function among this family and propose the reasonable improved classification of DyP-type peroxidase family, that is, class P, I and V. This classification is rather sophisticated and accelerates the logical study for DyP-type peroxidase family.

**FEMS-0973**

**Microbial genomics, evolution, phylogeny**

**APPLICATION OF GENOMICS TO PROKARYOTIC SYSTEMATICS: THE GENUS RHODOCOCCLUS AS A CASE STUDY**

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**Background**

Prokaryotic systematics provides the framework for all research in microbiology. Just as 16S rRNA gene sequence analysis revolutionised prokaryotic systematics a generation ago, genome sequence analyses now have the power to be a similarly disruptive technology. Case studies are needed to highlight the power of the new methodological approaches.

**Objectives**

To apply whole genome sequence analyses to reappraise the taxonomy of genus *Rhodococcus*.

**Methods**

We have determined genome sequences for 15 strains belonging to the genus *Rhodococcus*. Using this data and publicly available sequences, we have analysed genome sequences of 83 strains of 20 species within the genus *Rhodococcus* using phylogenomic, genetic and functional distance-based approaches.

**Conclusions**

Based on our analyses the rhodococci were resolved into six robust lineages that were as distant from each other as they were from other genera including representatives of *Nocardia* and *Gordonia*. These analyses also revealed a widespread problem of strain misidentification using classical approaches. The lineage containing *Rhodococcus rhodochrous*, the type species of the genus, represents the genus *Rhodococcus sensu stricto*. However, the others can potentially be designated as novel genera. *Rhodococcus equi* formed a distinct lineage with *Rhodococcus defluvii* consistent with our recent proposal that the former should be reclassified as "*Prescottella equi*". These data provide a case study demonstrating that genome sequencing is a cost effective and powerful tool for resolving complex taxonomic questions.

## FEMS-2321

### Microbial genomics, evolution, phylogeny

#### THE PLASMID COMPLEMENT OF LACTOCOCCUS LACTIS NCDO712

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#### Background

The lactic acid bacterium *Lactococcus lactis* NCDO712 was isolated from a dairy starter culture in the 1950s and it is the ancestor of the prophage and plasmid cured model strain MG1363. *L. lactis* NCDO712 was described to contain 5 plasmids (~50 kb, 13.6 kb, 8 kb, 3.8 kb, 2.7 kb) of which so far only two have been sequenced.

#### Objectives

The characterization of *L. lactis* NCDO172 will help establishing it as a relevant model strain because of its functional proximity to commercially used cultures. In combination with the genetically highly accessible derivative MG1363, it will form a useful couple to study industrially relevant traits of *L. lactis*.

#### Methods

Total DNA of *L. lactis* NCDO712 was sequenced using Illumina and PacBio. Additional PCR reactions and Southern hybridizations were done to complete the assembly. This resulted in 5 circular plasmids and one linear plasmid contig and allowed a SNP comparison with MG1363.

#### Conclusions

We here sequenced the complete plasmid complement of strain NCDO712, which resulted in the identification of 6 plasmids (55.4kb, 8.6kb, 3.6kb, 2.1kb, 15.5kb, 51.7kb) - one more than initially described. Next to an extracellular protease gene and the genes necessary for lactose utilization, which are on the already sequenced plasmid pLP712, the other plasmid-encoded genes include those of a variety of IS-located transposases, two restriction modification systems (specificity subunits) and stress related genes. The sequencing of the total DNA of strain NCDO712 also allowed identifying of some chromosomal differences between the strains NCDO712 and MG1363.

**FEMS-1043**

**Microbial genomics, evolution, phylogeny**

**SEQUENCING AND ANNOTATION OF THE GENOME FROM THE FILAMENTOUS FUNGUS *ACREMONIUM CHRYSOGENUM*, THE PRODUCER OF THE BETA-LACTAM ANTIBIOTIC CEPHALOSPORIN C**

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**Background**

*Acremonium chrysogenum* is the industrial fungal producer of the  $\beta$ -lactam antibiotic cephalosporin C, which is an important drug due to its broad activity against Gram-positive and Gram-negative bacteria. The source material for the modern chemically modified cephalosporin C derivatives is still mainly produced by *A. chrysogenum*, which marks its importance for biotechnology and medicine.

**Objectives**

Therefore it is of high interest to gain a deeper insight into the biosynthetic processes involved in cephalosporin C production and their regulation. Already established molecular tools like homologous recombination and the FLP/FRT marker recycling system are essential for the work with this fungus. A further crucial step for effective scientific research with *A. chrysogenum* is to disclose its genomic sequence.

**Methods**

DNA sequencing, sequence assembly, annotation

**Conclusions**

After quality trimming and assembly we were able to obtain a genome sequence with an overall length of 28.6 Mb distributed on 542 scaffolds. Furthermore 8901 protein coding genes were annotated using the protein data from closely related species and the UniProtKB/Swiss-Prot database. The potential to produce a variety of secondary metabolites is underlined by 42 putative secondary metabolite cluster found in the genome sequence. The available genomic sequence and annotation of *A. chrysogenum* represents a versatile platform for future functional genomic approaches (1).



1. **Terfehr D, Dahlmann TA, Specht T, Zadra I, Kürnsteiner H, Kück U.** 2014.  
Genome sequence and annotation of *Acremonium chrysogenum*, producer of the  $\beta$ -  
Lactam antibiotic cephalosporin C. *Genome Announc* **2**.

**FEMS-2684**

**Microbial genomics, evolution, phylogeny**

## **IN SILICO STRATEGIES FOR THE IDENTIFICATION AND CLASSIFICATION OF BACTERIAL AUTOTRANSPORTER PROTEINS**

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### **Background**

Autotransporters are the representatives of the type V secretion system proteins that are assembled into the outer membrane of bacterial cells, and are the most prevalent of the seven types of secretion systems in gram-negative bacteria.

### **Objectives**

The huge data from genome sequencing studies provides a resource of autotransporter proteins as well as pose a challenge in the identification and classification of the autotransporter sequences. We interrogate the bioinformatics strategies to address questions that increase our understanding of sequence-structure-function relationships in autotransporter proteins.

### **Methods**

By using the properties of the whole sequence we propose a new classification system for autotransporter proteins has been proposed. A Support Vector Machine model was subsequently constructed for the identification and classification of new FAEs into the pre-assigned clusters.

### **Conclusions**

This study centers on the descriptor-based classification and structural analysis of experimentally verified and putative autotransporter proteins; nevertheless, the framework presented here is applicable to every poorly characterized enzyme family.

**FEMS-1311**

**Microbial genomics, evolution, phylogeny**

**TEMPERATURE-INDUCED MUTAGENESIS AND MORTALITY IN CUPRIAVIDUS METALLIDURANS STRAINS**

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**Background**

*Cupriavidus metallidurans* is a  $\beta$ -proteobacterium which can be found in industrialized and anthropogenic environments. It was previously observed that growth of *C. metallidurans* CH34 at 37°C in rich media leads to high mortality (TIMM).

**Objectives**

The aim of this study is: (i) to scrutinize TIMM-inducing media and (ii) to investigate this phenomenon at the genetic and molecular level.

**Methods**

First, TIMM-induction was analyzed with different growth media. Next, the TIMM-phenotype was studied using a time-lapse microscope. Finally, whole-genome sequence analyses were performed for two derivatives with inherited resistance to all tested TIMM-inducing media.

**Conclusions**

The individual amino acids phenylalanine and cysteine and high ammonium concentrations could activate TIMM at 37°C in *C. metallidurans* CH34. Moreover, a mineral medium with low salt concentrations, i.e. Schatz-medium, also induced a high mortality at 37°C. These observations led to the hypothesis that different TIMM-inducing triggers are responsible for the phenotype. First, uptake of specific amino acids can lead to a toxic imbalance. Second, the TIMM-phenotype could be linked with osmotic potential and membrane integrity as characteristics like cell elongation, reproduction halting and cell lysis were induced. Interestingly, counteractivating agents were found as induction was inhibited by increasing the osmotic potential of Schatz-medium or by adding sorbitol to the different TIMM-inducing media. Both observations provided support for the hypothesis that membrane integrity plays a crucial role in the TIMM-phenotype. A common missense mutation was identified in a gene coding for an alcohol dehydrogenase. To examine the effect of this mutation complementation studies are currently being performed.

**FEMS-1101**

**Microbial genomics, evolution, phylogeny**

## **AN UNUSUAL STRAIN WHICH PROBABLY BE AN ANCESTOR IN THE EVOLUTIONARY PROCESS OF YERSINIA**

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### **Background**

We had isolated an unusual *Y. enterocolitica* strain from *Rattus norvegicus*, the brown rat. The strain did not utilize urea where API 20E identified *Escherichia coli*; however it grew well at 25°C where *E. coli* does not. We deduced the strain was *Y. enterocolitica* using sequence alignment of the 16 S rRNA gene.

### **Objectives**

To analysis the discrepancy with other *Y. enterocolitica* and comprehend its location in the evolutionary process of *Yersinia* by sequencing the whole genome of LC20.

### **Methods**

The LC20 genome was sequenced using a whole-genome shotgun sequencing strategy and Illumina Hiseq sequencing technology.

### **Conclusions**

Whole-genome comparison confirmed LC20 was *Y. enterocolitica*, the whole genome was collinear with *Y. enterocolitica* 8081. LC20 carried a great number of predicted CDSs and IS elements. Two plasmids shared low genetic homology with pYV from the *Yersinia* genus, one was an ancestral *Yersinia* plasmid and the other was novel encoding a number of transposases. The core-based and pan-based phylogenetic trees showed LC20 was classified into *Y. enterocolitica* cluster but on a distinct branch, which was closer to *Y. pestis* and *Y. pseudotuberculosis*. Some pathogenic and non-pathogenic *Y. enterocolitica* specific genes coexist in LC20. The urease gene did not exist on the genome consistent with API 20.

LC20 is sub-classified into *Y. enterocolitica* based at the level of the whole genome,

though its bacterial identification coding system of biochemical metabolism showed it was *E. coli*. The strain may be a progenitor of *Y. enterocolitica* and represent even an ancestor in the evolutionary process of *Yersinia* genus.

**FEMS-0947**

**Microbial genomics, evolution, phylogeny**

**CHARACTERIZATION OF A NOVEL SULFATE-REDUCING BACTERIUM  
POSSESSING DESULFOVIRIDIN AND VIBROID MORPHOLOGY, BELONGING  
TO THE FAMILY DESULFOMICROBIACEAE**

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**Background**

The order *Desulfovibrionales* is organized in four families; *Desulfovibrionaceae*, *Desulfonatronumaceae*, *Desulfohalobiaceae* and *Desulfomicrobiaceae*. The genus *Desulfomicrobium* is a sole member of family *Desulfomicrobiaceae* at the time of writing. The genus have been characterized as rod- or ellipsoidal-shaped morphology and absence of desulfovridin. In this study, a novel strain Pf12B<sup>T</sup> related to *Desulfomicrobium* species was isolated in pure culture.

**Objectives**

The aim of our study is characterization of the strain Pf12B<sup>T</sup>.

**Methods**

Strain Pf12B<sup>T</sup> was obtained from an enrichment culture established under sulfate-reducing conditions at 45°C with an inoculum of sediment from a brackish meromictic lake. Isolation of the strain was carried out by repeated agar shake dilution method.

**Conclusions**

Cells were vibroid, and motile. The closest relative of the strain was *Desulfomicrobium baculatum* with 16S rRNA gene sequence similarity of 91%. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that the strain Pf12B<sup>T</sup> belonged to the family *Desulfomicrobiaceae*, in the order *Desulfovibrionales*, *Deltaproteobacteria*. This result was also supported by the phylogenetic analysis based on DsrA amino acid sequence. Sulfate, sulfite, thiosulfate were used for electron acceptors. Formate and fumarate were used for substrate in the presence of sulfate. Yeast extract enhanced growth of the strain. The isolate grew at temperatures between 13-50°C with an optimum temperature range of 42-45°C. The strain was desulfovridin positive. Major cellular fatty acids of the isolate were C<sub>16:0</sub> and C<sub>18:0</sub>. It was suggested that the strain Pf12B<sup>T</sup> is a representative of novel species of novel genus within the family *Desulfomicrobiaceae*.

**FEMS-3010**

**Microbial genomics, evolution, phylogeny**

**CRYSTAL STRUCTURE OF ANABAENA SP. DYP-TYPE PEROXIDASE GIVES AN INSIGHT INTO SUBCLASSIFICATION OF DYP-TYPE PEROXIDASE FAMILY**

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**Background**

Dye-decolorizing peroxidase (DyP)-type peroxidase is a new family of heme peroxidase, distributing from bacteria to fungi. This name derives from the degradation ability of anthraquinone dyes. DyP-type peroxidases are known to show the similar structure. On the other hand, this family includes many proteins which show very low amino acid sequence similarity, resulting in inaccurate sequence alignment. This family has been subdivided into four classes (A, B, C, and D) based on the amino acid sequences. Recently, we reassessed the sequence alignment of DyP-type peroxidases using structure-based sequence alignment. The generated alignment reflected the differences hidden in the similar structures. Based on this result, we proposed the new subclass into three classes (P, I, and V). Here, classes P, I and V mean former classes B, A, and C with D, respectively. The structures of proteins of former classes C and D are almost same.

**Objectives**

*Anabaena* sp. DyP-type peroxidase (AnaPX) has been classified into different class (C or D) by different researchers. Therefore, it is expected that structure-based sequence alignment including the structure of AnaPX gives an insight into the subclass, especially, class V.

**Methods**

We determined the structure of AnaPX by X-ray crystallography and generated phylogenetic tree using MATRAS program (T. Kawabata, *Nucleic Acids Res.* **31**, 3367–3369 (2003)) as multiple three-dimensional alignment tool.

**Conclusions**

AnaPX showed the almost same structure as another structures belonging to class V and belonged to class V branch. This result supports our proposition that the border between former classes C and D is vague.

**FEMS-2405**

**Microbial genomics, evolution, phylogeny**

**GENOMIC EPIDEMIOLOGY OF MULTIPLE ACINETOBACTER BAUMANNII OUTBREAKS IN A VETERINARY INTENSIVE CARE UNIT**

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**Background**

*Acinetobacter baumannii* is an emerging pathogen primarily found in health-care associated settings. Due to its potential to acquire and control antimicrobial resistance and its ability to survive for extended periods in the environment, outbreaks are a threat and challenge for health care. Molecular epidemiology of *A. baumannii* is difficult because typing methods often lack resolution to identify transmission chains. Whole genome sequencing (WGS) forms an alternative method to investigate whether repeated outbreaks are independent events or are linked.

**Objectives**

Investigation into the relatedness of two outbreaks of *A. baumannii* in the companion animal intensive care unit (calCU) at Utrecht University in 2012 and 2014.

**Methods**

Multi Locus Sequence Typing (MLST) was performed for both outbreaks. All outbreak strains, three European Control strains (EC-I, -II and -III) and four environmental strains were sequenced on an Illumina Miseq platform. Genomes were assembled using SPAdes, horizontal gene transfer was detected using ClonalFrame, time-measured phylogeny was reconstructed using BEAST.

**Conclusions**

MLST of the isolates revealed that all animal isolates from both outbreaks in the calCU belong to ST2. Isolates from the outbreaks had similar resistance phenotypes, suggesting that the events are linked. Time-measured phylogeny on WGS data of the clinical isolates, reference isolates and public genome sequences revealed however, that the outbreaks are two independent events and that isolates from both outbreaks diverged at least 20 years before. Our study shows the benefit of WGS in outbreak management in a clinical setting for pathogens for which traditional molecular typing methods lack resolution.



**FEMS-1057**

**Microbial population genetics**

**IDENTIFICATION AND CHARACTERIZATION OF PATHOGENIC TYPES OF ESCHERICHIA COLI ISOLATES FROM A COHORT OF 1 TO 24 MONTH-OLD CHILDREN**

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**Background**

Colonization by Enterobacterias takes place in the first hours of life. This event is influenced not only by the surrounding environment, but also by bacterial flora of those in contact with the newborn, and the host conditions. The kind of bacterial flora acquired in this stage of life could influence the development of the newborn in a decisive form.

**Objectives**

To study Enterobacterial colonization process in a cohort of newborns through the identification of the presence of *Escherichia coli* enteropathogenic strains.

**Methods**

Coproscopic cultures of a cohort of newborns were carried out for a period of 24 months where bacterial genus and species were identified through conventional techniques. Enteropathogenic *E. coli* strains were re-identified using Enterotest and were later characterized by Multiplex PCR to identify different types of diarrheogenic *E. coli* and the presence of characteristic genes of each type.

Results. We identified diarrheogenic strains in the samples of the children from the first month of life. Majority of the strains were enteroaggregative (EAEC) type followed by Enterodifuse (DAEC) type which occupied the second place. We did not find association between the types of maternal diarrheogenic *E. coli* and that of the children. Interestingly, the identification of the diarrheogenic types did not have relationship with the presence of diarrhea in the subjects all along the period.

**Conclusions**

The colonization by Enteropathogens seems to be a "normal" event in the studied community without association with diarrheic episodes.

**FEMS-1058**

**Microbial population genetics**

**INITIAL IDENTIFICATION AND CHARACTERIZATION OF STRAINS OF  
KLEBSIELLA PNEUMONIAE ISOLATES FROM A COHORT OF CHILDREN OF 1 -  
24 MONTHS OLD**

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**Background**

Bacterial colonization takes place in the first hours of the birth of a baby. Until now, there is a consensus on the fact that newborns are contaminated by the maternal flora, principally when birth is by normal delivery. The Enterobacterias rapidly colonize the gastrointestinal tract, adapt and remain for prolonged periods, usually throughout the life of the individual.

**Objectives**

To follow-up the process of bacterial colonization and adaptation using *Klebsiella pneumoniae* as a model.

**Methods**

64 strains of *K. pneumoniae* isolated from 16 pairs of mother-child during a period of 24 months were studied. Bacterial identification was carried out through conventional techniques while bacterial identity was confirmed by Enterotest system. Also, antibiotic sensitivity tests were performed. Bacterial strains were genotypically characterized using RAPD-PCR and PFGE. PAST Program was employed for the identification of similar groups while the presence of 4 genes that codify bacterial adhesins were identified by means of PCR.

Results. We identified a series of *K. pneumoniae* clones in the children that are not related with the maternal strains. Two colonization patterns by *K. pneumoniae* were found. However, we did not find an association of these patterns with the way of birth (normal or cesarean delivery). It was found that along the period studied, there was population exchange of *K. pneumoniae* in the children studied.

**Conclusions**

In the case *K. pneumoniae*, the colonization of the children is not apparently from their mother and the way of their delivery does not influence in the colonization patterns.

**FEMS-2309**

**Microbial population genetics**

**NATURALLY OCCURRING VARIANTS OF INVASION FACTORS AND  
POLYHOSTALITY OF LISTERIA MONOCYTOGENES**

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**Background**

The Gram-positive bacterium *Listeria monocytogenes* causes a serious disease in humans and a wide range of animals. Cell invasion is a critical step of listerial infection. Bacterial proteins of internalin family InlA and InlB are critical for invasion of normally non-phagocytosing cells. The previously found correlation between InlA and InlB sequences and a host organism suggested that some naturally occurring variants of invasion factors might facilitate infection of certain hosts for the strains carrying these variants thus providing *L. monocytogenes* polyhostality.

**Objectives**

To test the suggestion that naturally occurring variants of *L. monocytogenes* invasion factors provide higher tropism to certain hosts.

**Methods**

Isogenic recombinant strains were obtained by cloning of *inlB* alleles found in human and animal isolates into the *L. monocytogenes* EGDe $\Delta$ inlB strain. To exclude regulatory effects, *inlBs* were put under the control of the EGDe P<sub>inlAB</sub> promoter and their secretion was driven by a heterologous signaling peptide. Human cells Hep-2 and Hek293 and murine cells C26 were used for the invasion assay.

Totally 8 *inlB* alleles were cloned from human and animal isolates into the same genetic background. The VIMHA004 and the VIMPHk23 *inlB* alleles provided the highest invasion of human and murine cells, respectively. The VIMHA004 and VIMPHk23 variants differed by substitutions Q72H in the N-terminal CAP-domain, N73S, V91I, I138L, P164L in the receptor-binding LRR-domain, T251M in the IR-domain.

**Conclusions**

*L. monocytogenes* InlB variants carrying substitutions in the receptor-binding domain LRR and the CAP and IR-flanking domains demonstrated different efficiency of invasion of human and murine cells.

**FEMS-1679**

**Microbial population genetics**

**MOLECULAR RISK ASSESSMENT OF SHIGA TOXIN-PRODUCING *E. COLI* O157:H7 STRAINS IN BELGIUM**

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**Background**

*E. coli* O157:H7 is one of the most feared zoonotic pathogens that can cause life threatening human disease such as the haemolytic uremic syndrome (HUS). Food sources of animal origin especially cattle, are the main cause of infection but many genotypes exist within this serotype of which their contribution to the clinical outcome of infection in humans is not well known.

**Objectives**

1-Determine the frequencies of distribution of different genotypes among *E. coli* O157:H7 strains recovered from food and human clinical sources using a combination of molecular subtyping methods and assess whether specific genotypes are overrepresented in human clinical strains. 2- Determine the potential correlation of genotype clusters with severe clinical symptoms.

**Methods**

A diverse collection of non-clonal human ( $n=100$ ) and food ( $n=70$ ) isolates from Belgium was characterized by conducting different molecular assays that have previously demonstrated non random distribution of *E. coli* O157:H7 genotypes among clinical and non-clinical isolates: lineage-specific polymorphism assay, Shiga-toxin-encoding bacteriophage insertion site assay, clade typing, *Tir* SNP assay, and variant analysis of Shiga toxin 2 and antiterminator Q genes. Genetic clustering of all data was performed using MCMC model.

**Conclusions**

Our results supported the epidemiological evidence of existence of clinically significant microbial genetic markers that are overrepresented among *E. coli* O157:H7 clinical isolates and which are important determinants of human infection risk. Furthermore, genetic clusters 1 and 3 were observed to be more frequently isolated from bloody diarrhea and haemolytic uremic syndrome, respectively. Our results may contribute to monitoring of genotypes with high virulence/transmission potential.

**FEMS-1998**

**Microbial population genetics**

**DEVELOPMENT OF MICROSATELLITE DNA MARKERS FOR LACHANCEA THERMOTOLERANS**

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**Background**

*Lachancea thermotolerans* is an important component of the grape/wine ecosystem and one of the first non-*Saccharomyces* yeasts recently commercialised as starter culture in winemaking. Therefore, there is an emerging need for a robust, rapid, and cost effective typing system to be used in ecological and technological studies.

**Objectives**

The complete genome sequence of the *Lachancea thermotolerans* was examined for development of variable number tandem repeat (VNTR) markers suitable in detecting genetic polymorphisms and discriminating among genotypes.

**Methods**

The Tandem Repeats Finder program was applied to detect microsatellites within the *L. thermotolerans* CBS 6340 genome ([www.genolevures.org](http://www.genolevures.org)). After an initial evaluation of potential VNTR markers using standard PCR and agarose gel electrophoresis, 10 markers were retained for further evaluation. Forward primers were labeled with different fluorescent dyes and PCR products were run in an ABI 3730 XLs DNA Analyser.

**Conclusions**

Ten polymorphic VNTR markers were analyzed using 48 *L. thermotolerans* isolates from geographical distant vineyards in Greece. These markers have been carefully selected to be widespread in different chromosomes, so as to be appropriate for phylogenetic analysis. They show a high degree of polymorphism with multiple alleles easily scored, some of which even on agarose gels. Thus, they are quite promising for investigating within and among population variability.

This work is co-financed by the European Regional Development Fund (ERDF) of the EU and by National Resources under the Operational Program Competitiveness and Entrepreneurship (EPAN II), Action "COOPERATION 2011".

**FEMS-2472**

**Microbial population genetics**

**GENETIC INSIGHTS INTO VEGETATIVE COMPATIBILITY GROUPING IN VERTICILLIUM: CHALLENGING THE MYTHS**

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**Background**

*Verticillium dahliae* is a notorious phytopathogenic ascomycete that causes vascular wilt in hundreds of plants worldwide. Classification of isolates into Vegetative Compatibility Groups (VCGs) has been widely used over the past 3 decades as a convenient way to describe population structures and predict pathogenicity potential. This method is based on the general assumption of genetic isolation between VCGs; heterokaryon formation is thus considered possible only between members of the same VCG.

**Objectives**

To test the traditional hypothesis of inter-VCG genetic isolation in *V. dahliae* through genetic investigations and phylogenetic analyses.

**Methods**

(i) Complementation tests using nitrate-nonutilizing (*nit*) and other mutant types of *V. dahliae* isolates, either by the standard procedure performed on minimal medium agar or by a novel high-throughput method in liquid microcultures. (ii) Microscopic investigations of self- and non-self heterokaryon formation between isolates labeled with nuclear fluorescent proteins. (iii) Phylogenetic analysis of a *V. dahliae* collection comprising members of all VCGs, using a highly polymorphic sub-region of the ribosomal IGS region.

**Conclusions**

True heterokaryotic reactions can be forced between *V. dahliae* members of different VCGs by using appropriate mutant types and assay conditions. These heterokaryons are frequently unstable, with uneven distributions of genetically dissimilar nuclei tending to segregate into novel homokaryotic mycelia. Most importantly, inter-VCG heterokaryon formation implies the absence of strict genetic barriers between *V. dahliae* VCGs and/or subgroups. These findings are consistent with the results of phylogenetic analyses of VCGs, which demonstrate a lack of correlation between the VCG classification system and intra-specific genetic groups.



**FEMS-2588**

**Microbial population genetics**

**GENETIC POPULATIONS ANALYSIS OF XYLELLA FASTIDIOSA FROM CITRUS AND COFFEE PLANTS SYMPATRICALLY GROWN IN SAO PAULO STATE, BRAZIL**

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**Background**

In Brazil *Xylella fastidiosa* (*Xf*) bacteria are responsible by diseases in economically important crops like sweet orange causing the Citrus Variegated Chlorosis and coffee causing the Coffee Leaf Scorch. Population-structure-based studies focusing on the systemic-colonizer, vector-dependent, multi-host plant pathogenic bacterium *Xf* has provided remarkable information about their ecology and biology, specially for the citrus infecting *Xf* populations. In contrast, no information is currently available about the genetic structure of *Xf* populations in coffee and uncertain information are known about the cross infection in citrus and coffee plants by *Xf*.

**Objectives**

To determine the genetic structure of *Xf* populations infecting coffee trees both the genotypic and genetic diversity. Also, assay using cross infection between coffee and citrus strains were realized. Finally, we also like to understand how both hosts and agricultural management has shaped the ecology of both *Xf* populations.

**Methods**

We sampled symptomatic coffee plants from geographic regions sympatric to citrus plantations in Sao Paulo State, Brazil, and isolated hundreds isolates from each region. This isolates were genotyped using 14 genomic microsatellite markers.

**Conclusions**

The coffee-associated *Xf* populations had higher gene diversity and allelic richness than citrus, typical of an evolutionarily older population. Even sharing the same vectors and geographic area *Xf* from coffee and citrus are genetically and biologically different populations with no admixture of strains. *Xf* from coffee were geographically structured similarly as the sympatric populations from citrus. Cross infection were successful only in coffee plants inoculated with *Xf* from citrus, but no colonization were observed after 13 months.

**FEMS-0707**

**Microbial proteomics**

## **L-ASPARTIC ACID CONSUMPTION AS A BASE FOR AMINO ACIDS AND THEIR DERIVATES PRODUCTION BY CORYNEBACTERIUM GLUTAMICUM**

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### **Background**

Corynebacteria are Gram-positive Actinobacteria inhabitants of diverse ecological niches. The most industrial-interesting species is *Corynebacterium glutamicum*, which is involved in amino acids production as pharmaceutical products and mainly as food/feed enhancers. This bacterium produces per year more than 2.93 million tons of L-glutamic acid and 1.95 of L-lysine. However, the coryneform bacteria entered the 21st century with a refocusing of their industrial point of view. Thus, relevant bioproducts such as chemicals and biodegradable polymers, as well as ingredients or additives in food, feed, cosmetics and pharmaceuticals have extended the portfolio of *Corynebacterium* [e. g.: cadaverine].

### **Objectives**

The aspartate pathway is responsible for the biosynthesis of a high number of metabolites of biotechnological importance such as L-lysine, L-threonine, L-methionine and L-isoleucine, as well as other close related compounds: the amino acid L-valine, the vitamin D-pantothenate or the biogenic amine cadaverine (1,5-diaminopentane). The last one is the proof-of-concept compound of the ERA-IB 3<sup>rd</sup> joint call supported project named SCILS (<http://www.era-ib.net/scils>).

### **Methods**

*C. glutamicum* is able to use different alternative nitrogen compounds as L-glutamine or creatinine, but little is known about the possibility of L-aspartic acid utilization. The 2D-DIGE analysis results, as well as a microarrays study of an aspartate-consumption defective mutant, have shown that *C. glutamicum* is able to use L-aspartic acid as nitrogen source.

### **Conclusions**

The bioconversion to different amino acids, which can be the precursor of industrial relevant biomolecules (e.g.: L-lysine decarboxylation yields cadaverine), is the main explanation of the aspartate use.



**FEMS-2264**

**Microbial proteomics**

**A BLUEPRINT OF SULFUR METABOLISM FROM THE PROTEOME OF HYDROGEN-PRODUCER THERMOCOCCUS ONNURINEUS NA1**

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**Background**

The hyperthermophilic archaeon, *Thermococcus onnurineus* NA1, has been shown to produce biohydrogen (H<sub>2</sub>) when using CO, formate, or starch as a growth substrate. This strain can also utilize elemental sulfur (S<sup>0</sup>) as a terminal electron acceptor for heterotrophic growth and reduce it to H<sub>2</sub>S.

**Objectives**

To gain insight into sulfur metabolism, the proteome of *T. onnurineus* NA1 cells sampled under sulfur culture conditions was quantified and compared with those under formate, CO and starch culture conditions.

**Methods**

Using label-free nano-UPLC-MS<sup>E</sup>-based comparative proteomic analysis, we found that approximately 38.4 % of the total identified proteome (589 proteins) were significantly up-regulated (≥1.5 fold) under sulfur culture conditions.

**Conclusions**

Many of these proteins are functionally associated with carbon fixation, Fe-S cluster biogenesis, ATP synthesis, sulfur reduction, protein glycosylation, protein translocation, and formate oxidation. Our results also revealed markedly lower expression levels of enzymes involved in the sulfur assimilation pathway, as well as cysteine desulfurase under sulfur culture condition. The present results provide a first global atlas of the proteome changes triggered by sulfur. This study will contribute to a better understanding of the mechanisms and physiology of *T. onnurineus* NA1 cells in response to sulphur.

**FEMS-0687**

**Microbial proteomics**

**PROTEOGENOMICS OF THE NUTRITIONALLY VERSATILE, MARINE, SULFATE-REDUCING BACTERIUM *DESULFOCOCCUS MULTIVORANS***

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**Background**

Sulfate-reducing bacteria play a major role in marine carbon and sulfur cycling. Members of the metabolically versatile deltaproteobacterial *Desulfococcus/Desulfosarcina*-cluster are abundant in marine sediments and therefore of ecophysiological importance. A metabolically versatile representative of this cluster is *Desulfococcus multivorans* that is able to completely oxidize a variety of organic acids, including fatty acids up to C<sub>14</sub>, as well as aromatic compounds under anoxic conditions.

**Objectives**

Proteomics-enhanced genome annotation of *D. multivorans* was based on cells adapted to 17 different substrate conditions and aimed at metabolic reconstruction and deciphering the regulatory network.

**Methods**

Manual revising of automatically predicted and annotated protein-coding sequences (CDS) was combined with proteomic data (2D-DIGE, shotgun, membrane protein-enriched fraction) of substrate-adapted cells to allow for improved functional prediction.

**Conclusions**

The closed genome sequence consists of a 4.45 Mb circular chromosome with 3946 predicted CDS. The metabolic versatility of *D. multivorans* is reflected by the high number (269) of encoded proteins involved in organic carbon catabolism, 156 of which were detected. Out of the 324 genomically predicted transport proteins 104 could be detected in the membrane protein-enriched fraction. The presence of >200

genes encoding proteins involved in signal transduction should facilitate from the basis of a regulatory network for adapting to changing environmental conditions. Accordingly, out of 1247 identified proteins, only 220 were present under all analyzed substrate conditions, unravelling a high level of substrate-dependent regulation in *D. multivorans*. Overall, the study underpins the value of proteomic analysis to enhance functional genomic predictions, providing new perspectives on our metabolic understanding of sulfate-reducing bacteria.

**FEMS-0681**

**Microbial proteomics**

**PROTEOMIC ANALYSIS OF THE *PENICILLIUM CHRYSOGENUM* B13  
ENZYMATIC COCKTAIL FOR LIGNOCELLULOSIC BIOMASS  
SACCHARIFICATION**

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**Background**

Fungi are known as active degraders of lignocellulosic materials in nature, soil, compost and forest topsoil. The method for degradation depends on the fungus genus with different results. The key for their degradation capacity resides in the fact that the extracellular enzymes have different capacities more or less active according to the lignocellulosic substrate. These enzymatic extracts are used in biotechnological processes in the industry.

**Objectives**

*Penicillium chrysogenum* B13 strain (CTM2012-32026 project<sup>1</sup>) is an overproducer of feruloyl esterase (FAE) able to hydrolyze the ester bonds between the hydroxycinnamic acids and the plant cell wall polysaccharides. As a result of FAE activity, the polysaccharides are more accessible, allowing complete digestion by the rest of the enzymes involved in the plant cell wall degradation.

**Methods**

*P. chrysogenum* B13 strain was grown in presence of sugar beet pulp and the enzymatic extract secreted to the culture medium was obtained at the time of greatest FAE activity. Two-dimensional gel electrophoresis of proteins was performed and subsequently proteins were identified by means of a MALDI-TOF mass spectrophotometer system.

**Conclusions**

The analysis of the results showed that the FAE activity is increased by 9.27% with respect to the wild strain

*P. chrysogenum* Wisconsin 54-1255 and that 42.22% of the secreted proteins are

enzymes associated with the degradation of plant cell wall, compared to only 11.59% when the culture medium does not contain plant substrate.

<sup>1</sup>The project CTM2012-32026 is funded by Spanish Ministry of Economy and Competitiveness (MINECO) through the Subprogramme for Non-Guided Fundamental Research Projects. 2012.



**FEMS-1310**

**Microbial proteomics**

## **IDENTIFICATION OF REDOX PARTNER OF HELICOBACTER PYLORI HP0377 PROTEIN.**

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### **Background**

*Helicobacter pylori* is the common human pathogen colonizing the gastric epithelium of humans with severe consequences. HP0377 is a Dsb thiol oxidoreductase which acts as CcmG protein, involved in the maturation of apocytochrome c. CcmG proteins are kept in the reduced form by an integral membrane protein DsbD or its shortened analogue, CcdA. Both proteins catalyze the transfer of electrons from cytoplasmic thioredoxin across the inner membrane to the periplasm. *H. pylori* does not encode DsbD, however it encodes homologue of CcdA (HP0265).

### **Objectives**

The aim of this study was to establish whether HP0265 is responsible for HP0377 rereducing.

### **Methods**

To inactivate *hp0265*, the allelic exchange strategy was used. A recombinant plasmid pUWM2019 (based on a vector non-replicating in *Helicobacter* cells) constructed by a two step PCR method, was used for mutagenesis. It contains the *hp0265* gene disrupted by insertion of a kanamycin resistance cassette into the gene coding sequence. We then tested whether deletion of *hp0265* affects the redox state of HP0377 using AMS trapping strategy. This agent can only modify covalently free thiols, resulting in a molecular mass increase of about 490 Da.

### **Conclusions**

We found that *H. pylori* HP0377 is rereduced by HP0265. Obtaining the *hp0265* mutant was dependent on presence of the reducing agent, DTT. Moreover, the growth of the *hp0265* mutated strain without the presence of DTT was much slower than the growth of wt strain, which indicates the importance of HP0265 and its reducing activity for bacterial survival.

**FEMS-2355**

**Microbial proteomics**

**A METAPROTEOMIC APPROACH FOR ANALYSIS OF MICROBIAL COMMUNITY STRUCTURE AND FUNCTION FOR IMPROVEMENT OF BIOGAS PLANT PERFORMANCE**

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**Background**

To avoid downtime in operation and to further optimize operation of biogas plant (BGP) profound understanding of the composition and function of microbial community converting the biomass to methane is required.

**Objectives**

Our main goal is to understand how process conditions determine the process performance, the taxonomic and functional composition of the microbial community and vice versa. For such investigations, the quantification of the composition of the active community is essential. In particular, the presence of key enzymes of metabolic pathway correlates well with the community activity. Thus, the present study investigated the metaproteome of 40 industrial-scale BGPs.

**Methods**

The applied metaproteome workflow involved protein extraction using liquid phenol, tryptic digestion, peptide separation by liquid chromatography coupled to tandem mass spectrometry (Velos Orbitrap Elite), and data analysis with the *MetaProteomeAnalyzer* software [1].

**Conclusions**

For each BGP, about 500 proteins, covering the main metabolic pathways of the biogas process, namely hydrolysis, fermentation, acetogenesis and methanogenesis, were identified. The microbial communities detected by metaproteome analysis of the BGPs clustered according process temperature (mesophilic and thermophilic) [2,3], and substrate composition.

Application of metaproteomics allowed the acquisition of profound knowledge about community structure and function of microbial communities and contribute to our understanding of the conversion of biomass into methane. This knowledge could be used to improve monitoring and control of BGP, and to support development of new BGP designs.



**FEMS-0763**  
**Microbial proteomics**

**IDENTIFICATION OF PEPTIDASES GENERATING CONSTITUTIVE  
COLLAGENOLYTIC ACTIVITY OF GEOBACILLUS THERMOLEOVORANS  
DSM15325**

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**Background**

While the main biological importance of collagen degradation for prokaryotic pathogens is tightly connected with virulence, thermophilic bacteria use collagenolysis when exploit collagen for nutrition. To date the view that collagen catabolism by thermophilic bacteria is a result of a cascade of synergistically active inducible peptidases is prevailing. Usually this model of collagen degradation omits the thermophilic bacteria constitutively produced collagenolytic peptidases. The constitutive collagenolytic activity of thermophilic bacteria was never deliberately analyzed and the understanding of constitutive peptidases importance for collagen hydrolysis *in vivo* is obscure, what reduces the overall fundamental understanding of collagenolysis biological roles for prokaryotes.

**Objectives**

Detect hydrolytic activity of *Geobacillus thermoleovorans* DSM15325 constitutively produced collagenolytic peptidases. Identify detected hydrolases by mass spectrometry.

**Methods**

*G. thermoleovorans* DSM15325 was cultivated in modified M9 medium up to late exponential growth phase when the total secretome proteins were precipitated and analyzed by SDS-PAGE. The evaluation of collagenolytic peptidases activity was performed by gelatin zymography and azocollolysis. Detected peptidases were excised from acrylamide gel and subjected to mass spectrometry analysis. Obtained enzyme sequences were analyzed *in silico*.

**Conclusions**

The Bacillolysin (protein ID AEV20496.1), Pz-peptidase A (protein ID BAD99433.1) and B (protein ID BAD99434.1) were identified as peptidases generating constitutive collagenolytic activity of *G. thermoleovorans* DSM15325.



**FEMS-2614**  
**Microbial proteomics**

## **THE MEMBRANE PROTEOM OF CRONOBACTER SAKAZAKII, MALONATICUS AND TURICENSIS**

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### **Background**

Bacteria from genus *Cronobacter* are opportunistic pathogens associated with rare but severe infections mainly among neonates. Based on the epidemiological studies and molecular typing methods such as MLST is obvious that *C. sakazakii*, *C. malonaticus* and *C. turicensis* are prevalent in neonatal infections and particularly sequence types ST4, ST7 and ST19 are considered as an attribute of virulence strains.

### **Objectives**

The aim of this study was to map membrane of virulent strains being as the gateway to the host organism and to contribute to the knowledge of virulence features of this genus.

### **Methods**

We applied several techniques for membrane proteins isolation such as the whole cell membrane isolation by phenol extraction method, the membrane sub-fractionation into outer membrane, periplasm and inner membrane, and the immunoprecipitation using polyclonal antibodies against whole *Cronobacter sakazakii* cells. The isolated proteins were consequently identified by mass spectrometry and peptide mass fingerprinting method.

### **Conclusions**

Membrane proteins play a pivotal role in bacterial pathogenesis thus a complex study of membrane proteome of pathogens is invaluable. Here we present membrane proteomes report of the strains considered as pathogenic focusing on virulence traits determination.



**FEMS-1542**

**Microbial proteomics**

**ANALYSES OF ACTIVATED SLUDGE METAPROTEOMES OF A WASTEWATER TREATMENT PLANT DURING WINTER AND SUMMER PERIOD**

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**Background**

Molecular biological approaches (i.e. community fingerprinting, metagenome sequencing) revealed that only a small portion of the microbial community typically found in wastewater treatment plants (WWTP) can be cultivated in the laboratory. In order to understand its function without isolating single strains, metaproteomics analysis has been applied to activated sludge samples.

**Objectives**

The impact of changes in temperature (winter and summer period) on metaproteomes of activated sludge samples derived from a full-scale WWTP was investigated to enable a detailed description on functional level.

**Methods**

For reduction of sample complexity prior to MS analysis, different fractionation techniques including reversed phase liquid chromatography (RP-LC), sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), and liquid isoelectric focusing were combined and compared regarding effort and quality of resulting protein identifications. The derived spectra were identified using the UniProtKB/Swiss-Prot protein database. Additionally, identified KEGG ontologies (Kyoto Encyclopedia of Genes and Genomes) and Enzyme Commission numbers were used to plot proteins hits into pathway maps of the central carbon and nitrogen metabolism.

**Conclusions**

As expected, the high resolving but most time consuming three-dimensional approach yielded the highest amount of protein identifications. The results revealed taxonomic differences in the abundance of specific classes, such as Beta- and Gammaproteobacteria, in activated sludge collected during winter and summer period. Fractions of eukaryotic proteins decreased from winter to summer period, indicating a better degradation performance during summer period due to increasing process temperature. Additionally, more proteins associated to the nitrogen



metabolism, like hydroxylamine dehydrogenase or ammonia monooxygenase, were identified in summer period.

**FEMS-2163**

**Microbial proteomics**

## **CALCIUM-TRYPTOPHAN INTERACTIONS IN CALCIUM-BINDING PROTEINS**

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### **Background**

Calcium ion is the important structural element in bacterial cells and is also involved in cell signaling. Also, the calcium binding may enable protein folding and enzymatic activity. Aromatic amino-acids in proteins can stabilize the native protein structure via pi-pi interactions and may also play a role in calcium binding by cation-pi interactions. Terbium (Tb<sup>3+</sup>) is the phosphorescent lanthanide that may be used as a calcium binding analog. Tb<sup>3+</sup> increases the quantum yield as it binds to calcium binding sites (in proteins, DPA), which enables spectroscopic ion binding measurements. In our previous studies of FrpC protein of *Neisseria meningitidis* we found the calcium binding as a driving force for FrpC folding. The two Trp residues of FrpC also play an important role as stabilizing factor in calcium binding and enzymatic activity, the auto-catalytic cleavage.

### **Objectives**

In order to estimate the importance of calcium-Trp interactions in proteins, we analyzed PDB crystal structures possessing both Trp residues and bound calcium.

### **Methods**

In all calcium-containing-protein-structures in PDB (n=6403) relative Ca/Trp positions were analyzed (392000 cases). FrpC studies: Steady-state fluorescence spectroscopy.

### **Conclusions**

We confirmed that in calcium-binding enzymatic domain of FrpC, calcium is bound in the close proximity to Trp residues that supports the calcium-Trp interaction. Using PDB database search, we found that such calcium-Trp position is rather rare among protein structures and that calcium is most frequently positioned toward nitrogen hetero-atom in indol.

**FEMS-1440**

**Microbial proteomics**

**QUANTITATIVE SHOTGUN PROTEOMIC APPROACH FOR THE STUDY OF S.COELICOLOR M145 AND S.LIVIDANS TK24, TWO PHYLOGENETICALLY CLOSELY RELATED STRAINS WITH VERY DIFFERENT ABILITIES TO PRODUCE ANTIBIOTICS**

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**Background**

*Streptomyces* are filamentous Gram positive soil bacteria of industrial interest due to their ability produce two-thirds of all known antibiotics as well as other so-called "secondary metabolites" useful to human health or agriculture. A reverse correlation between the content in TriAcylGlycerol (TAG) and the ability to produce antibiotics was recently demonstrated. In the presence of glucose, the lipid content of *S. coelicolor* is less than 10% its dry cell weight (DCW) indicating that its TAG content is very low and abundantly produces antibiotics whereas the total lipid content of *S. lividans* is up to 30% its DCW and does not produce antibiotics. Interestingly, in the presence of glycerol, the two strains have the same lipid content (about 30% of their DCW).

**Objectives**

In order to understand the molecular basis of these phenotypical differences between these two *Streptomyces* species, we developed a novel gel-free shotgun proteomic approach.

**Methods**

This strategy involves a simple protein extraction followed by proteolysis in presence of trypsin and LysC and LC-MS/MS analysis by Q-exactive MS (Thermo Fisher). We performed a three-factor analysis of variance that included: strain factor (*S.lividans/S.coelicolor*); carbon source factor (glucose/glycerol), and time factor (36h/48h/72h).

**Conclusions**

More than 2500 proteins were identified corresponding approximately to 30% of the genome. This study showed different pattern of expression of genes of central carbon and lipids metabolism as well as antibiotics biosynthesis, upon growth on glucose or glycerol. These results revealed some potentially interesting genes to be genetically modified for further improvement of antibiotics and/or TAG production by *Streptomyces* species.

**FEMS-0989**

**Microbial proteomics**

**THE SMALL PROTEIN SCO2038 CONTROLS STREPTOMYCES COELICOLOR DIFFERENTIATION BY MODULATING TRYPTOPHAN BIOSYNTHESIS**

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**Background**

In *Streptomyces coelicolor* amino acid metabolism is an important clue of the morphological and physiological differentiation program and, differently from other bacteria, the expression of amino acid biosynthetic genes is not subjected to end-product negative regulation. In some amino acid biosynthetic gene clusters, such as tryptophan, histidine and proline, small orfs (about 100-300 nucleotides) were identified. These small orfs, such as sco2038, encode proteins whose cellular role have to be elucidated to highlight possible novel and crucial molecular mechanisms controlling amino acid synthesis and, thus, differentiation program.

**Objectives**

The aims of this work are:

1. the understanding of the effects exerted by tryptophan on primary metabolism, morphological differentiation and antibiotic production;
2. the study and characterization of the SCO2038 function as modulator of tryptophan biosynthesis.

**Methods**

- Differential proteomic analysis based on 2D-DIGE and MS procedures.
- SEM analysis.
- Generation and characterization of sco2038 mutants
- Identification of potential SCO2038 interaction partners by pull down assay coupled with MS identification and Bacterial Adenylate Cyclase Two Hybrid System.
- qRT-PCR analysis.

**Conclusions**

The obtained results revealed that tryptophan controls the expression of metabolic and regulatory proteins and promotes aerial mycelium formation, spores production and actinorhodin antibiotic biosynthesis. Moreover, the small orf sco2038, encodes a 7 KDa protein playing a key role in modulating tryptophan biosynthesis and thus, morphological differentiation. In the light of these results we propose to rename sco2038 as *trpM*, the gene encoding the tryptophan biosynthesis Modulator TrpM.

**FEMS-2585**

**Microbial proteomics**

## **SLP PROFILES IN ENDEMIC AND NON-ENDEMIC CLOSTRIDIUM DIFFICILE PCR-RIBOTYPES**

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### **Background**

*Clostridium difficile* is divided into approximately 400 PCR-ribotypes, some of them are widespread and associated with severe disease. *C. difficile* PCR-ribotypes 014/020 and 002 are the most common among endemic PCR-ribotypes.

Many bacteria, including *C. difficile*, express surface layer protein (Slp). This protein forms surface-exposed proteinaceous layer which mediates interactions with the external environment, i.e. adhesion to host cells as in case of *C. difficile*. Two Slp proteins with molecular weights (MWs) of 32-38 kDa (low MW) and 42-48 kDa (high MW) are expressed and vary in size in different *C. difficile* strains. Slp typing is described, but not widely used.

### **Objectives**

The aim of the study was to compare slp profiles of two endemic PCR-ribotypes (014/020 and 002) and selection of non-endemic PCR-ribotypes.

### **Methods**

Eighty-eight *C. difficile* isolates from 21 different PCR-ribotypes were included. Extraction of Slp proteins was performed by using low-pH glycine solution and further analyzed by SDS-PAGE.

### **Conclusions**

Five different slp profiles (1-5) were identified. Slp profiles 1 and 2 were the most frequent and present in the majority of PCR-ribotypes. Endemic and non-endemic

PCR-ribotypes shared 3 out of 5 slp profiles. Two remaining slp profiles were present only in one strain each.

Our results indicate that slp profile does not contribute significantly to *C. difficile* colonization properties and endemicity.

**FEMS-0673**

**Microbial proteomics**

## **FLUORESCENT BIOMARKERS FOR MEMBRANE SEPARATION**

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### **Background**

The aim of the project is to develop biomarkers to help distinguish between the inner and the outer membrane of *Escherichia coli*. The fluorescent proteins mCherry, *E. coli* Flavin binding Fluorescent Protein and superfolder GFP, were used as biomarkers. These were directed to the inner or outer membrane using signal sequences from lipoproteins or by fusion with membrane proteins. The fusion partners were the outer membrane protein A (OmpA), the artificial TAT-lipobox signal sequence for transport to and lipid anchoring in the outer membrane, the major coat protein of phage pf3 that is inserted into the inner membrane, and mistic, a integral inner membrane protein from *Bacillus subtilis*.

### **Objectives**

The aim of the project is to develop a bacterial strain (*E.coli* BL21 DE3) with optimized expression levels of the fluorescent markers for co-localization studies of bacterial membrane proteins.

### **Methods**

The methods used in the project include molecular biology (cloning, PCR), microbiological (culturing, recombinant protein expression) and biochemical (cell fractionation, membrane isolation) techniques. In addition, the project will also use analytical methods such as fluorometry and fluorescence microscopy.

### **Conclusions**

The fluorescent marking of the two membranes makes membrane fractions easier to detect after membrane separation. It also help to assess the quality of the separation procedure.



**FEMS-2159**

**Microbial proteomics**

**CHARACTERISATION OF THE BSA TYPE THREE SYSTEM SECRETOME OF BURKHOLDERIA PSEUDOMALLEI USING HYPER-SECRETING MUTANTS**

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**Background**

Many Gram-negative bacteria utilise Type III secretion systems (T3SS's) to deliver effector proteins into target host cells where they hijack cellular processes for their own benefit. T3SS's form an injectosome with proteins spanning both membranes of the bacterium and an external needle through which effector proteins are delivered into the target cell. *B. pseudomallei*, the causative agent of the tropical disease melioidosis in humans and animals, possesses three distinct T3SS's, of which the Bsa system has been shown to play a role in invasion of non-phagocytic cells, escape from the endocytic compartment and virulence in murine models of melioidosis. To date few proteins have been proven to be secreted by the Bsa apparatus and, by analogy with other pathogens that deploy T3SS's, it is likely that there are many other effectors awaiting discovery

**Objectives**

To characterise the total secretome of *B. pseudomallei* in standard laboratory media and the repertoire of Bsa-secreted effector proteins.

**Methods**

We have determined the effector secretome of the Bsa T3SS using hyper-secreting mutants of coupled with iTRAQ, a gel free quantitative proteomics technique.

**Conclusions**

Our study provides one of the most comprehensive core secretomes of *B. pseudomallei* described to date and identified 26 putative Bsa-dependent secreted proteins that may be considered candidate effectors.

**FEMS-0760**

**Microbial proteomics**

**THE EXCRETORY/SECRETORY PROTEOME OF TRICHOMONAS VAGINALIS  
INDUCED BY NEUTROPHIL**

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**Background**

The protist parasite *Trichomonas vaginalis* causes one of the most common non-viral sexually transmitted disease in humans, trichomoniasis. Previous studies indicate that neutrophils, leukotriene B<sub>4</sub> (LTB<sub>4</sub>) and interleukin-8 (IL-8) are found in the vaginal discharges and vaginal smears of patients infected with *T. vaginalis*.

**Objectives**

IL-8 is often associated with inflammation, also known as neutrophil major chemotactic factor, causing them to migrate toward the site of infection by *T. vaginalis* and start innate immune response to defense. Previous studies focused on the profiling of chemoattractants from neutrophils stimulated by *T. vaginalis* or *T. vaginalis* ESPs, but very little is known about the cell-cell interaction between *T. vaginalis* and neutrophil. We hypothesize that both *T. vaginalis* ESPs and direct contact with neutrophil can induce the release of chemoattractants in the vagina.

**Methods**

In this study, we confirmed that both *T. vaginalis* and ESPs can induce neutrophil to release IL-8. We also used 2-Dimensional SDS-PAGE to investigate the differentially expressed ESPs from *T. vaginalis* before and after contact with neutrophil. A total of 16 differentially expressed protein spots were identified by MALDI-TOF-MS, and discussed the possible roles of identified proteins in the biology of host-parasite interactions.

**Conclusions**

We established a representative 2-DE map of ESPs of *T. vaginalis* induced by neutrophil and identified differentially expressed protein that may promote cytoadherence and long-term colonization while ESPs from neutrophil may be related to implicate in the first line of host defense.

**FEMS-1650**

**Microbial proteomics**

**COMPARATIVE PROTEOMICS ANALYSIS FOR THE IDENTIFICATION OF TARGETS OF THE TMK1 MAP KINASE IN THE MYCOPARASITIC FUNGUS TRICHODERMA ATROVIRIDE**

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**Background**

Mycoparasitic species of the filamentous fungus *Trichoderma* are commercially applied as biological control agents against fungal plant pathogens. The mycoparasitic interaction of *Trichoderma* with the host fungus comprises specific recognition events leading to activation of "molecular weapons" in the mycoparasite which are involved in host attack and lysis and result in utilization of the host fungus as a nutrient source for the mycoparasite.

Investigations on the underlying intracellular signal transduction pathways of *Trichoderma atroviride* revealed the involvement of a Mitogen-activated protein kinase (MAPK) which is essential for triggering the mycoparasitic Response. Mutants missing the *tmk1* gene show reduced mycoparasitic activity against host fungi.

**Objectives**

The aim of the presented study was the identification of target proteins being regulated by the Tmk1 MAPK pathway upon host recognition. To this end, *T. atroviride* wild-type and the delta-*tmk1* mutant were co-cultivated with the host fungus *Rhizoctonia solani* and *Trichoderma* mycelia were harvested from the confrontation zone upon direct interaction between the two fungi and from respective un-induced (self-confrontation instead of confrontation with the host) controls.

**Methods**

Two-dimensional Difference Gel Electrophoresis (2-D DIGE) was applied for comparatively analysing the proteomes of the four samples with the aim to find mycoparasitism-relevant targets of the Tmk1 MAPK.

## Conclusions

Bioinformatic analyses revealed 60 proteins being regulated in the wild-type response (WT vs. *R. solani* minus WT vs. WT) and 126 proteins being host-induced Tmk1 targets (WT vs. *R. solani* minus delta-*tmk1* vs. *R. solani*), of which 30 and 60 proteins, respectively, were identified by mass spectrometry.

## **FEMS-3009**

### **Microbial proteomics**

#### **PROTEOMICS ANALYSIS OF THE *B. SUBTILIS* INNER SPORE MEMBRANE**

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### **Background**

The endospore is the dormant form of *Bacillus subtilis* and many other Firmicutes. By sporulation these spore formers can survive very harsh physical and chemical conditions. Yet they need to go through germination to return to their growing form. The spore inner membrane (IM) has been shown to play an essential role in triggering the initiation of germination; however, its protein composition was not comprehensively characterized yet.

### **Objectives**

To isolate IM from *B. subtilis* spores and to characterize the *B. subtilis* spore IM proteome.

### **Methods**

The spores were chemically decoated and enzymatically deprived of the cortex. Subsequently the core was mechanically disrupted and the IM fraction was collected by differential centrifugation. Tryptic peptides, acquired from in-gel digestion, were analyzed using Ion trap LC-MS/MS, which led to identification of the spore IM proteins.

### **Conclusions**

We have adapted the spore IM isolation protocol to proteomics studies. Over 900 proteins could be identified from the *B. subtilis* spore IM preparations, in which ca. one-third were predicted to be membrane proteins. In addition to the previously known IM proteins, a number of novel IM proteins were identified which are likely to provide new insights into the IM protein composition, its functions and in particular into the spore germination machinery.

**FEMS-1371**

**Mobile genetic elements**

**PARTIAL NUCLEOTIDE SEQUENCE OF THREE RESISTANCE PLASMIDS OF PSYCHROTOLERANT ENTEROBACTERIA ISOLATED FROM FILDES PENINSULA, KING GEORGE ISLAND (MARITIME ANTARCTICA)**

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**Background**

The presence of plasmids carrying class 1 integrons in a collection of psychrotolerant enterobacteria isolated from Fildes Peninsula was previously established. In these plasmids, we could identify a DNA region with three ORFs (*intl1*, *dfrA14* and *hyp*, encoding a hypothetical protein) highly similar to a fragment originally identified in plasmid pKOX105 from *Klebsiella oxytoca* (HM\_126016).

**Objectives**

DNA sequence analysis of plasmids purified from some of these enterobacterial isolates.

**Methods**

Plasmid DNA from isolates CDTR5, CN11 and HP19 were purified with Gene Plasmid Midprep Elute® HP (Sigma-Aldrich Co, MO) and sequenced in a Ion 314™ Chip on a Ion PGMTM System (Life Technologies,) at the IIBCE. The reads were assembled using CLC Genomics Workbench v6.5 (CLC bio). Annotation of pHP19 contigs and comparison with plasmids pECL\_A and pKOX105 were done using RAST server (<http://rast.nmpdr.org/rast.cgi>).

**Conclusions**

Sequence analysis of pHP19 showed a region of 7181 nts highly similar (99% identity by Blastn) with a region of pKOX105 (54641pb). This contig includes the *intl1* gene and other ORFs encoding a DNA-cytosine methyltransferase and a probable membrane protein.

The sequences of other plasmidic regions were similar to those of *Enterobacter cloacae* ATCC 13047 plasmid pECL\_A (IncFII) (199562pb) (NC\_014107), including genes for replication, stability and conjugation. Other cluster genes related with mercury, arsenic, tellurite, nickel and copper resistance identified were identical to those found in pECL\_A. Thus this plasmid is likely a mosaic of portions of plasmids

that have been characterized in mesophilic enterobacteria, showing that HGT between these bacteria and the Antarctic microbiota is rather common.

**FEMS-1533**

**Mobile genetic elements**

## **C-DI-GMP CONTROL MODULES CONFINED WITHIN MOBILE ELEMENTS: THE CASE OF ICEAca<sub>TY.2</sub>**

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### **Background**

The intracellular nucleotide c-di-GMP has recently emerged as one of the central elements of the signal transduction network linking perception of environmental or intracellular cues to specific alterations in cellular function. In response to different input signals, the opposing activities of diguanylatecyclases (DGCs) and phosphodiesterases (PDEs) control the cellular concentration of c-di-GMP. Inside the cell, c-di-GMP binds a currently unknown number of effector molecules that subsequently interact with cognate target components to produce a variety of output phenotypes (e.g. transition from planktonic to biofilm). Given the multiplicity of c-di-GMP related genes in most bacterial genomes and their scattered distribution within the genomes, the elements that belong to the same c-di-GMP control module are difficult to uncover.

Recently, an integrative conjugative element -ICEAca<sub>TY.2</sub>- has been characterized in the extreme acidophile *Acidithiobacillus caldus* ATCC 51756. ICEAca<sub>TY.2</sub> is an actively excising element of widespread occurrence in a collection of *At. caldus* strains, that behaves as a cohesive heritable unit. Bioinformatic analysis of ICE.2-type elements has revealed that c-di-GMP synthesis/degradation functions and putative c-di-GMP binding effectors and targets co-occur within the element.

### **Objectives**

To gain insight into the participation of c-di-GMP genes encoded by ICEAca<sub>TY.2</sub> as a functional control module.

### **Methods**

To test this hypothesis the expression of the c-di-GMP genes encoded by ICEAca<sub>TY.2</sub> was quantified by real-time PCR under different growth conditions (e.g. planktonic, attached, stressed).

### **Conclusions**

Transcriptional behavior of key c-di-GMP module components in attached cells and upon DNA damaging conditions support the hypothesis and further suggest that this control module could be critical for biofilm development and DNA exchange.



**FEMS-0638**

**Mobile genetic elements**

## **ENVIRONMENTAL ISOLATES OF *V. PARAHAEMOLYTICUS* CARRY SXT/R391 INTEGRATIVE CONJUGATIVE ELEMENTS**

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### **Background**

SXT/R391 Integrative conjugative elements (ICEs) are self-transmissible mobile genetic elements able to confer adaptive features to bacterial hosts, including *Vibrionaceae*. They share a conserved genetic scaffold encoding their own conjugation, integration, excision, and regulatory machinery, interspersed with variable DNA clusters located in conserved hotspots.

### **Objectives**

*In silico* analysis of 96 *V. parahaemolyticus* genomes was performed to identify Integrative Conjugative Elements (ICEs) of the SXT/R391 family.

### **Methods**

Four novel ICEs were detected in environmental non-pandemic *V. parahaemolyticus* strains isolated in China, Spain, and Malaysia (2002-2008). ICE sequences were annotated using the RAST annotation pipeline and comparative analysis was performed with the Artemis Comparative Tool, BLASTN and BLAST-PSI. Given their unique genetic contents they were named: ICE *VpaSpa1* (~111 kb), ICE *VpaMal1* (~70 kb), ICE *VpaChn4* (~66 kb), ICE *VpaChn5* (~86 kb). We identified gene sequences from other ICEs as well as unique genetic features such as a set of heat-shock proteins and chaperones likely to respond to environmental stress (ICE *VpaSpa1*); genes with transpeptidase domains belonging to the beta-lactamase TEM family (ICE *VpaSpa1*); putative type 1 restriction/modification systems (ICE *VpaChn5*, ICE *VpaSpa1*); type III restriction-modification systems (ICE *VpaMal1*); *ars* operon encoding an efflux system mediating arsenic resistance (ICE *VpaMal1*, ICE *VpaChn4*); and an antibiotic resistance cluster carrying *floR*, *strAB* and *sul2* genes (ICE *VpaChn5*).

### **Conclusions**

*V. parahaemolyticus* ICEs exhibit significant genetic polymorphisms, sign that the structure of each ICE has been shaped by frequent recombination, with genetic

content likely dependent on the specific locale in the environment from which it was originally isolated.

**FEMS-1251**

**Mobile genetic elements**

## **OBTENTION OF HYPERACTIVE INTEGRASES BY EVOLVING SYNONYMOUS GENES**

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### **Background**

Integrans are gene recruitment platforms that allow for rapid bacterial evolution, playing a major role in the acquisition of antimicrobial resistance genes. The integrase catalyzes the reaction between *attC* and *attI* sites. However, its activity is finely tuned to preferentially process *attI* x *attC* and *attC* x *attC* reactions, as they allow for the acquisition and rearrangement of cassettes, rather than the *attI* x *attI* reaction. Being a less frequent phenomenon, the structural basis of *attI* recognition has yet remained elusive.

### **Objectives**

We have conducted directed evolution experiments on the integrase to obtain hyperactive mutants for the *attI* x *attI* reaction. In order to explore a broader evolutionary landscape of the integrase we have recoded the protein into two alternative, yet synonymous, alleles of the *intI1* gene.

### **Methods**

In a first round of experiments we obtained among the three alleles 8 mutations conferring an increase in recombination rates. We reinserted all mutations into the three wt alleles, obtaining a 100-fold increase in recombination efficiency. Re-evolving these alleles yielded new mutants with recombination rates for *attI* x *attI* comparable to those of the wild type protein for the *attI* x *attC* reaction. Along these experiments we obtained landscape-specific mutations and the re-evolution of a codon to a residue beyond its evolutionary landscape in any of the three starting alleles.

### **Conclusions**

We have successfully explored an enlarged evolutionary landscape of the integrase allowing us to obtain hyperactive molecules, while shedding light on the structural features that are important for the recognition of the *attI* site.



**FEMS-2726**

**Mobile genetic elements**

**CONJUGATIVE TYPE IVB PILUS RECOGNIZES LIPOPOLYSACCHARIDE  
STRUCTURE OF RECIPIENT CELLS TO INITIATE PAPI-1 PATHOGENICITY  
ISLAND TRANSFER IN PSEUDOMONAS AERUGINOSA**

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**Background**

Horizontal gene transfer is an important mechanism in *Pseudomonas aeruginosa* for adapting to a wide range of environments and, in particular, for acquiring virulence determinants which are mostly carried on pathogenicity islands. One of the largest pathogenicity island of *P. aeruginosa* is PAPI-1, a 108-kb island which is known to be transferred from a donor to a recipient strain through a type IVb pilus. However, the PAPI-1 acquisition mechanism has not been elucidated to date.

**Objectives**

We aimed at investigating the mechanism of PAPI-1 acquisition in recipient cells.

**Methods**

To determine the receptor for conjugative type IVb pilus, a standard PAPI-1 transfer assay described by Carter et al. (2010) was carried out using PA14TnC2 as a donor and 38 PAO1 mutants with an altered lipopolysaccharide (LPS) biosynthesis pathway as recipients. The loss of receptor for conjugative type IVb pilus in PAO1 mutants can result in a significant decrease of PAPI-1 transfer. The conjugative pilus receptor was then confirmed with a competition assay by adding outer-membrane and LPS preparations into the standard mating assay.

**Conclusions**

Transfer efficiency of PAPI-1 to the *AlgC* mutant producing a truncated LPS core was reduced by three orders of magnitude compared to that of wild-type PAO1, indicating that LPS acts as a receptor for conjugative type IVb pilus. The homopolymer of D-rhamnose, or rhamnan structure was confirmed as a specific receptor for conjugative type IVb pilus. Further investigation will define if *P.aeruginosa* that have acquired

PAPI-1 specify a surface exclusion mechanism by LPS modification preventing further recognition by conjugative pilus.

**FEMS-2000**

**Mobile genetic elements**

**REGULATION OF THE MOBILITY OF THE LVH-GENOMIC ISLAND OF  
LEGIONELLA PNEUMOPHILA**

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**Background**

Horizontal gene transfer (HGT) and mobile genetic elements (MGEs) are major generators of genome plasticity and diversity. Certain of these elements can exist in the *Legionella pneumophila* (*Lp*) genome in an excised or integrated form and encode for type IV secretion systems (T4SS) and/or conjugation systems. Intriguingly, each of these elements encodes a homologue of CsrA (carbon storage regulator). CsrA is a global regulator of virulence in *L.p*. The Lvh-region is one of these MGEs encoding a T4ASS and a CsrA homologue.

**Objectives**

Deciphering the mechanisms of mobility of the Lvh

**Methods**

Distribution of the Lvh was analyzed by comparative genomics of 50 *L.p* strains, the mobility and regulation of transfer were analyzed by mutagenesis, conjugation experiments, Q-PCR, RNA-Seq and whole genome sequencing of the transconjugants

**Conclusions**

The Lvh mobility is mediated by a phage-like integrase. It is transferable at a rate of  $10^{-4}$  and inserts specifically in the *tmRNA* gene in all analysed transconjugants. Using a  $\Delta dotA$  strain we show that the Dot/Icm T4SS is implicated in the conjugation of the *lvh*-region, as the transfer rate drops to  $10^{-7}$ . Conjugation experiments in a  $\Delta ihf$  mutant (Integration host factor), a nucleoid-associated protein revealed its role in the mobility of the Lvh. Most interestingly the putative RNA-binding protein LvrC (CsrA homologue) affects the mobility of Lvh by conjugation in specific growth conditions. Thus, The mobility of the predicted self-transmissible Lvh-genomic island is regulated by a complex network of global (IHF) and specific regulatory proteins (LvrC) and depends largely on the Dot/Icm T4SS

## **FEMS-2660**

### **Mobile genetic elements**

#### **STRUCTURE AND PROPERTIES OF PLASMIDS ISOLATED FROM ANCIENT ACINETOBACTER LWOFFII STRAINS, THEIR ROLE IN HORIZONTAL GENE TRANSFER OF ANTIBIOTIC AND HEAVY METAL RESISTANCE GENES**

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### **Background**

Three ancient *Acinetobacter lwoffii* strains were aseptically isolated from East-Siberian permafrost sediments and carbon-14-dated to 15.000-30.000 years and 2-3 million years before present.

### **Objectives**

Investigate the structure and properties of plasmids from ancient *Acinetobacter lwoffii* strains, and the presence of various heavy metal and antibiotic resistance genes on those plasmids.

### **Methods**

Complete genomic sequences (average coverage >30) of three strains were obtained using pyrosequencing method. 13 plasmid sequences were assembled and manually analysed using bioinformatics software and public databases and verified with PCR amplification of regions where ambiguous results occurred.

### **Conclusions**

Each strain carried at least two plasmids with their sizes varied between 6 kb and 287 kb. Three largest plasmids designated pKLH208 (287 kb), pKLH211 (190 kb) and pKLH220 (130 kb) belonged to ED23-35, ED45-23 and VS15 strains respectively. Each of these plasmids encoded similar replication and segregation systems and 12 to 18 IS-elements of IS1, IS4, IS5, IS6 and IS66 families with almost twice as many IS-elements with interrupted *tnp* genes, suggesting frequent transposition processes. Apart from two different mercury resistance operons found only on pKLH208 and pKLH211, large plasmids encoded operons conferring resistance to cobalt, zinc, cadmium, chrome and arsenical compounds, several copies of copper and iron intake regulating operons, urea degrading operons, *aadA* (streptomycin/spectinomycin) and *tetA-tetR* (tetracycline) antibiotic resistance genes. We found that 11 of 13 identified plasmids, including two large plasmids pKLH208 and pKLH220, encoded either



mobilisation or conjugation and type IV secretion systems and are potentially capable of transferring horizontally.

**FEMS-1677**

**Mobile genetic elements**

**COMPARISON OF INTEGRON ATT<sub>C</sub> SITES: THEIR STRUCTURAL FEATURES DIFFERENTIALLY INFLUENCE THE FREQUENCY AND ORIENTATION OF CASSETTE INTEGRATION**

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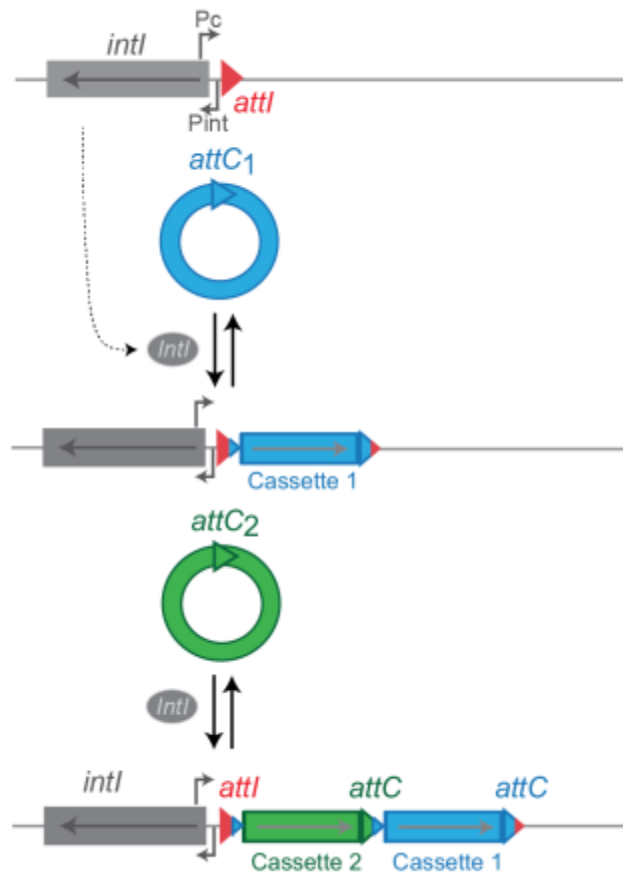
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**Background**

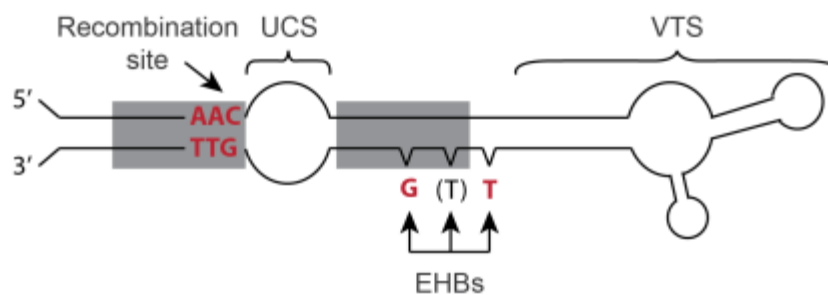
The integron is a bacterial recombination system for capturing and rearranging gene cassettes through site-specific recombination, which plays a role in the rise of antibiotic multiresistance in Gram-negative bacteria. The distinctive property of integron recombination sites flanking the cassettes (*attC*) is that they have almost no sequence conservation. Instead, their single-stranded DNA possesses conserved structural features, and *attC* sites are recombined as a folded form of the bottom DNA strand. Contrary to the bottom strand, the recombination of the top strand would integrate the cassette in the opposite orientation, preventing the expression of its gene from the promoter provided by the integron platform.

**Objectives**

## The integron



## Schematic representation of an attC site



In order to understand the basis of this specificity, we tested the recombination of several *attC* sites, and selected 3 of them for further analysis.

## Methods

For these sites, we mutated their structural features and quantified the changes in frequency and orientation of cassette integration for the corresponding bottom and top strands.

## **Conclusions**

We observed that some of the structural features, such as the extrahelical bases (EHBs), play similar roles for all of the tested sites, but the extent to which they influence the frequency and the orientation of cassette integration differs among *attC* sites. Yet other structural features, such as the unpaired central spacer (UCS), have very different, sometimes even opposing effects depending on the *attC* site. Our study suggests that the structural requirements for *attC* site recognition are rather relaxed, and that the control of cassette integration might be governed by more complex mechanisms than previously assumed.

**FEMS-2161**

## **Mobile genetic elements**

### **POSSIBLE ROLE OF A MOBILE GENETIC ELEMENT IN TRANSLATIONAL MODULATION**

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### **Background**

A consortium of microorganisms adapted to grow in acidic environments participate in the bioleaching of minerals. The facultative aerobic bacterium *Acidithiobacillus ferrooxidans* is a Gram negative, chemolithotrophic, autotroph that utilizes either Fe<sup>2+</sup> or sulfur reduced compounds as electron donors.

### **Objectives**

We are interested in the role of non-coding RNAs (sRNAs and tRNAs) and RNases encoded in a mobile genetic element in translation control.

### **Methods**

By means of bioinformatic analyses 37 genes encoding tRNAs were detected in the mobile element ICEAfe1 from *A. ferrooxidans* strain ATCC 23270. Encoded tRNAs are aminoacylated *in vivo* in *A. ferrooxidans*, although they are transcribed at low levels.

Two strategies were used for their identification of sRNAs: 1. Bioinformatic prediction of transcription units in intergenic regions (IGR) of *A. ferrooxidans* genome and comparison to RNA deep sequencing data. 2. The identification of sRNAs that bind to the RNA chaperone Hfq were identified by RNA deep sequencing. Currently we are working in the validation of target mRNAs of the identified sRNAs. At least two sRNAs encoded in the ICEAfe1 were validated.

Four type II toxin-antitoxins systems specific for strain ATCC 23270 were identified in the ICEAfe1. All four toxins encoded an RNase that is counteracted by the

antitoxin

### **Conclusions**

Different genes encoding possible factors that modulate translation have been identified in the integrative conjugative element ICE *Afe1* from *Acidithiobacillus ferrooxidans*. Whether these systems effectively modulate translation in this organisms is currently under investigation.

**FEMS-1742**

**Mobile genetic elements**

## **A NEW SHUTTLE-EXPRESSION SYSTEM TO GENERATE POLYHISTIDINE-TAGGED FUSION PROTEINS IN STAPHYLOCOCCUS AUREUS AND ESCHERICHIA COLI**

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### **Background**

A better understanding of the metabolism of *S. aureus* is needed. However, only few tools are available for production of tagged fusion proteins.

### **Objectives**

To develop *S. aureus*-*E. coli* shuttle vectors for the expression of target genes and *rgs-his<sub>6</sub>* codon fusions.

### **Methods**

The strong constitutive promoter of *S. aureus* type 1 capsule biosynthetic gene 1A (*P<sub>cap</sub>*) upstream of a multiple cloning site (MCS) and codons for *rgs-his<sub>6</sub>* were synthesized and inserted into pBUS1 background. The MCS contained a BclI site to generate C-terminal RGS-His<sub>6</sub> translational fusions of cloned genes through a simple cut-religation method. Using PCR-based techniques, two vectors were constructed that contain the *P<sub>cap</sub>*-MCS-*rgs-his<sub>6</sub>* sequence (pBUS1-*P<sub>cap</sub>*-HC and pTSSCm-*P<sub>cap</sub>*) and two promoter-less variants suitable for cloning genes including their own promoter (pBUS1-HC and pTSSCm). The plasmids contained the *E. coli* origin ColE1, the terminator sequence *rrnB*(T1)<sub>5</sub> and the tetracycline resistance marker *tet*(L) for *S. aureus* and *E. coli*. Gram-positive replicon was improved through either complementation of the minimum pAMα1 replicon from pBUS1 with the single strand origin *oriL* from pUB110 or substitution with a pT181-family replicon. Plasmid stability, copy numbers and recombinant protein synthesis was analyzed in *S. aureus* and *E. coli*.

### **Conclusions**

The new shuttle vectors displayed increased copy numbers and segregational stability in *S. aureus*. Feasibility of *rgs-his<sub>6</sub>* codon-fusion, gene expression and protein purification were demonstrated in *S. aureus* and *E. coli* using the macrolide, lincosamide, streptogramin B resistance gene *erm*(44). The new His-tag expression

system represents a helpful tool for the direct analysis of target gene function in staphylococci.



## FEMS-2416

### Mobile genetic elements

## THE PRESENCE OF TEMPERATE BACTERIOPHAGES IN UROPATHOGENIC ESCHERICHIA COLI STRAINS

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### Background

Horizontal gene transfer vectors, including bacteriophages, promote the spread of a broad spectrum of genes, among them some encoding medically relevant traits such as virulence factors. In *Escherichia coli* (*E. coli*), temperate bacteriophages have been shown to promote the spread of shiga toxin genes across shigatoxigenic strains of the species<sup>1</sup>.

### Objectives

Here we aim to assess the presence of lysogenic bacteriophages in uropathogenic *E. coli* (UPEC) strains, and to investigate a possible correlation with the virulence factor profiles of the examined strains.

### Methods

Twenty UPEC strains were screened for the presence of 15 different virulence-associated traits (*fimH*, *papC*, *papGII*, *sfaDE\_focIC*, *afaBC\_draBC*, *hlyA*, *cnf1*, *usp*, *ibeA*, *iucD*, *iroCD*, *iroN*, *fyuA* and *ireA*) by PCR amplification of target genes, and were subsequently assigned virulence scores by counting possession of these genes. Additionally, strains were screened for the presence of lysogenic bacteriophages by induction with UV light. Data were analysed using Student's *t*-test.

### Conclusions

We have found that out of the 20 screened strains, nine possessed UV-inducible lysogenic bacteriophages. Furthermore, data analysis has shown that the strains harbouring UV-inducible lysogenic bacteriophages also possessed a significantly higher virulence score than the strains lacking UV-inducible bacteriophages ( $p < 0.01$ ). The results of this preliminary study imply that temperate bacteriophages might have a potential role in virulence factor transfer across UPEC strains.

### Reference

1. Smith DL, Rooks DJ, Fogg PC, et al. Comparative genomics of shiga toxin encoding bacteriophages. BMC Genomics, 2012, 13: 311.

**FEMS-2669**

**Mobile genetic elements**

**THE IMPACT OF Tn3-FAMILY TRANSPOSONS ON DIVERSITY AND EVOLUTION OF BACTERIAL MOBILE GENETIC ELEMENTS**

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**Background**

Transposable elements (TEs) are components of nearly all bacterial genomes. They promote structural changes in DNA, which may result in different phenotypes. We focused on the elements of the Tn3 family – the most abundant group of bacterial transposons.

**Objectives**

Our studies shed a new light on Tn3 family, which is much more diverse than previously thought. We showed that Tn3 elements comprise (i) a composite transposon generated by two copies of a non-composite cryptic transposon, as well as (ii) simple non-autonomous elements (TIMes - Tn3-derived inverted-repeat miniature elements) and (iii) their derivatives. We have provided for the first time evidence **that some of the non-autonomous transposons may contain, in their core regions, functional plasmid replication and stabilizations systems, which can be transferred among replicons co-residing in a bacterial cell.** The transposition events may therefore significantly influence structures of bacterial plasmids leading to generation of molecules exhibiting novel properties. Bioinformatic analyses of the nucleotide sequences of several bacterial genomes revealed the presence of large elements originating from the Tn3-family non-composite transposons, which acquired additional genetic information, thus forming a kind of transposable genomic islands.

**Methods**

Standard methods of molecular biology were used.

**Conclusions**

The obtained results indicate the powerful role of the Tn3-family TEs in the mobilization to transposition of segments of genomic DNA. Such shuffling of genetic information, may significantly influence the structures of bacterial genomes. Huge diversity of the identified elements allows to draw conclusions concerning the high dynamics of evolution of this group of TEs.

**FEMS-1655**

**Mobile genetic elements**

## **CULTURE-INDEPENDENT RECOVERY OF IS1216 COMPOSITE TRANSPOSONS FROM ORAL METAGENOMIC DNA.**

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### **Background**

A composite transposon is a transposon consisting of two insertion sequences (ISs) flanking a DNA segment which often carries antibiotic resistance (AR) genes. If both IS elements are the same then it is possible that one copy of the IS element plus the intervening DNA segment can excise as a circular form. This circular molecule has been called an "Unconventional Circularizable Structure" (UCS) or "Translocatable Unit" (TU). IS 1216 composite transposons have been shown to carry various AR genes. In addition UCSs have also been found containing IS 1216.

### **Objectives**

To screen for IS 1216 composite transposons and IS 1216 containing UCSs in human oral metagenomic DNA.

### **Methods**

PCR primers were designed to amplify in the outward direction from IS 1216. The IS 1216 PCR products were then cloned and analysed.

As our PCR strategy would also detect UCSs, PCR primers were designed to amplify outward from the intervening DNA region to determine if each IS 1216 amplicon was derived from a UCS circular structure.

### **Conclusions**

We found four different IS 1216 composite transposons and confirmed the presence of two novel UCSs in the oral metagenomic DNA. One of them contains an antiseptic resistance gene and one contains a universal stress response protein encoding gene.

This is the first report of a PCR strategy to amplify the DNA segment on the composite transposons in metagenomic DNA, and is also the first time that UCSs have been found in metagenomic DNA. This PCR strategy can be used to identify

novel AR genes associated with mobile genetic elements in metagenomes from any environment.

**FEMS-1667**

**Mobile genetic elements**

## **DETECTION AND CHARACTERISATION OF INTEGRON GENE CASSETTES IN HUMAN ORAL METAGENOMIC DNA.**

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### **Background**

An integron is a genetic element, which is able to capture gene cassettes and direct the expression of open reading frames (ORFs) contained within them. They are involved in the dissemination of antibiotic resistance genes (ARGs) in many pathogens. The oral cavity is a reservoir of ARGs; however, these have not been shown previously to be present on an integron cassette.

### **Objectives**

To investigate the presence of integrons and associated gene cassettes in the human oral cavity by using a PCR-based approach.

### **Methods**

Different primer sets were tested on oral metagenomic DNA obtained from both the UK and Bangladesh, including novel primers based on the *Treponema denticola* integrase and *attC* sequences, and previously published primers targeting various conserved regions on different integrons. The resulting metagenomic amplicon libraries were then sequenced and analysed.

### **Conclusions**

A diverse array of ORFs presents on gene cassettes and associated with integron integrases have been identified. The cassettes contain ORFs predicted to confer antibiotic resistance and adaption to environmental stresses including a putative chloramphenicol acetyltransferase, bleomycin binding protein, multidrug transporter, cof-like hydrolase, competence and motility related proteins.

This is the first study confirming the presence of integron gene cassettes in oral metagenomic DNA. The predicted proteins are likely to carry out a multitude of functions however the function of the majority is as yet unknown. We hypothesise that the oral cavity may be a rich source of diverse integron cassettes because it is such a variable physicochemical and stressful environment for its bacterial inhabitants.

## **FEMS-2530**

### **Mobile genetic elements**

#### **HUH TRANSPOSASES AND GENOME DYNAMICS**

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#### **Background**

We address the central role of a functionally related group of transposable elements called “HuH elements” in structuring and shaping bacterial genomes. We have identified and characterised a widespread class of IS (IS200/IS605 family) fundamentally different from classical TEs: they use obligatory single strand DNA intermediates and have ends with subterminal imperfect palindromes (IP) which are recognised and bound by their transposases. These transposases are members of a larger “HuH” endonuclease superfamily. The protein binds the subterminal IP located some distance from the cleavage sites. Remarkably, cleavage sites are not recognised directly by the protein but by short “guide” sequences 5' to the IP foot. Recognition involves a network of canonical and non-canonical base interactions similar to those found in RNA structures.

#### **Objectives**

We have demonstrated the importance of the lagging strand template for activity of some members and our *in silico* genomic analysis suggests that all IS200/IS605 family members have evolved a mode of transposition that exploits ssDNA at the replication fork.

#### **Methods**

Some members of this group appear to have been domesticated to perform important roles in the prokaryotic cell: such as homing endonucleases in some group I prokaryotic introns and as enzymes responsible for proliferation of short intergenic multicopy palindromic regulatory sequences called REPs.

#### **Conclusions**

The results provide an overview of the role of “HuH” transposases and their associated mobile elements in shaping the prokaryotic genome.

**FEMS-0657**

**Mobile genetic elements**

**FUNCTIONAL CHARACTERIZATION OF AN INOSITOL METABOLISM  
ENCODING GENE CLUSTER CONTAINED IN ICEEFM1 OF ENTEROCOCCUS  
FAECIUM**

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**Background**

Whole genome sequencing revealed the presence of an integrative conjugative element, *ICEEfm1* in a clinical *Enterococcus faecium* (Efm) isolate (E1504) with an insertion of a cluster of 10 genes putatively involved in inositol metabolism.

**Objectives**

Functional characterization of the inositol metabolism (*iol*) gene cluster.

**Methods**

A cre-lox recombination system was used to construct a markerless *iolD* deletion mutant in strain E1504. The phenotype was determined by growth on minimal medium with inositol as carbon source (M-ino). The genetic organization of the gene cluster was determined by operon and promoter mapping and expression was determined after growth in rich and minimal medium in the presence and absence of myo-inositol. An *in vivo* mouse gut-colonization model was used to compare colonization abilities of E1504-wildtype and *iolD* mutant.

**Results.** The *iolD* deletion resulted in abolished growth in M-ino. The gene cluster is organized as an operon of 10 genes with its own promoter. Expression was only observed in the presence of myo-inositol and absence of other carbon sources. No difference was observed in gut-colonization abilities between wild-type and mutant.

**Conclusions**

The 10 gene insertion in *ICEfm1* confers Efm the ability to grow when only inositol is available as carbon source. Due to the availability of additional other sugars the *iol* gene cluster did not provide a selective advantage in the *in vivo* mouse model. Possibly the gene cluster was acquired in an environment where carbon sources were limited and only inositol was present like e.g. plants and soil.



**FEMS-1731**

**Mobile genetic elements**

## **GENETIC AND LIFE-HISTORY TRAITS DETERMINING LYSOGENY AND THE DISTRIBUTION OF PROPHAGES**

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### **Background**

Bacterial viruses (phages) are ubiquitous and have a strong impact on microbial population dynamics. Temperate phages may become prophages upon cell infection, producing lysogenic hosts and thus contributing to the evolution of bacterial gene repertoires.

### **Objectives**

We identified and studied 2246 prophages among the genomes of 1196 bacterial species to pinpoint the genetic and life-history traits associated with lysogeny and how they shape the distribution of prophages among bacteria.

### **Methods**

Surprisingly, whereas lysogens have larger genomes and are more often pathogens, they are not different in terms of cell volume or defense systems, like CRISPR-Cas. Importantly, growth-related trade-offs seem to be key to the decision of lysogeny. We propose that lysogeny is more adaptive when hosts show large variations in the resources they can provide. Accordingly, bacteria with low minimal doubling times, which are associated with strong variations in growth rates and cell volume, show remarkably high rates of lysogeny. This fits theory suggesting that lysogeny is a strategy of slow vertical reproduction in waiting for future more efficient horizontal reproduction. This strategy has important consequences for bacterial evolution: we find that lysogens have much more diverse gene repertoires, after controlling for other factors, showing the key role of lysogeny in bacterial adaptation.

### **Conclusions**

Our work shows a complex dependency of bacterial genetic diversification on host-parasite interactions and the life-history traits of both bacteria and phage. It allows establishing coarse-grained predictions of the abundance and distribution of prophages and explains the paucity of prophages in certain bacterial clades.

**FEMS-1291**

**Mobile genetic elements**

**CONTRIBUTION OF INSERTION SEQUENCE ELEMENTS TO THE GENOME PLASTICITY OF CUPRIAVIDUS METALLIDURANS AE126**

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**Background**

Mobile genetic elements play a significant role in bacterial evolution. Insertion Sequence (IS) elements are of specific interest as they constitute an importing driving force for genome plasticity. *Cupriavidus metallidurans* AE126 (type strain CH34 cured of pMOL30) harbors 19 distinct IS elements from nine IS families, reaching a total of 52 intact IS copies. *C. metallidurans* AE126 displays in the presence of toxic zinc concentrations spontaneous mutations resulting in an increased zinc resistance.

**Objectives**

The objective of this study is: (i) to determine the contribution of IS elements to genome adaptation of AE126 after zinc stress and (ii) to examine the putative stress-specific inducible transposition of IS elements.

**Methods**

Zinc resistant AE126 derivatives were screened by PCR and mutations were identified by sequencing. Promoter transcription of IS elements was analyzed with the promoter probe vector pGLR1.

**Conclusions**

All derivatives had a base substitution/indel (45%) or an insertion of an IS element (55%) in the regulatory locus of *cnr* (cobalt-nickel resistance). Seven IS elements were identified to be transposed into the regulatory locus of *cnr*. Three elements were found more often with respect to their copy number and transcription of their promoters were induced by supplementing zinc or cadmium to the growth medium but not nickel or cobalt. Some AE126 derivatives, e.g. with an *ISRme5* element inserted in the same orientation as *cnrYXH*, are pre-adapted as they evolved to ameliorate resistance to other stress challenges. Pre-adaptation could putatively be explained by the increased transcription of the mutated *cnr*-operon and concomitant co-transcription of *ISRme5*.

**FEMS-3004**

**Mobile genetic elements**

**DISTRIBUTION AND VARIANT CHARACTERISTICS OF VSP-I ISLAND IN VIBRIO CHOLERAЕ**

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Objective To investigate the distribution and characteristics of vibrio seventh pandemic island-I (VSP-I) in *Vibrio cholerae*. Method All *V. cholerae* genome sequences in GenBank were downloaded and screened for VSP-I island using VSP-I (VC0175~VC0185) of strain N16961 as the reference sequence. MUMmer software was used to analyze single nucleotide polymorphism (SNP); OrthMCL software was used for homologous gene analysis; finally the BLAST software was used to search sequence variation and functional annotation. Conclusions VSP-I widely exists in the genomes of all the seventh epidemic *V. cholerae* O1 strains and its sequence is highly conserved; Among the 105 *V. cholerae* O1 strains, the VSP-1 sequences of 94 strains were completely identical, the VSP-I sequences of 11 strains had 1~14 SNP loci, respectively. The VSP-I exists also in the genomes of two non-O1/O139 strains. However, their VSP-I sequences contain some insertion and deletion (INDEL) comparing to that of O1 strains. VSP-I had 9 conserved genes totally. Among them, VC0183 existed in all strains, but its length of the non-O1/O139 strains was different to the O1 strains. Five (VC0181~VC0185) of 9 genes were involved in its transfer and integration. And VC0178 encoding potato glycoprotein analogues and VC0180 encoding protein hydrolase may be related to virulence. As a specific genetic cluster within the seventh cholera pandemic strains, VSP-I has been transferred to the non-O1/O139 strains, and variation of its sequence has been occurred as well.

**FEMS-2024**

**New antimicrobial mechanisms**

**DAMAGES CAUSED BY SILVER NANOPARTICLES AND SILVER NITRATE ON PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS**

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**Background**

The antimicrobial activity of silver is well documented, however its mechanism of action is not well understood. It is unclear whether the effects of silver nanoparticles (Ag-NPs) and Ag<sup>+</sup> ions are similar or have different antimicrobial mechanisms.

**Objectives**

The objective was to compare the damages caused by Ag-NPs and silver nitrate (AgNO<sub>3</sub>) on gram-positive and gram-negative bacteria by transmission electron microscopy.

**Methods**

Ag-NPs were synthesized by reduction of AgNO<sub>3</sub> with sodium citrate. *Pseudomonas aeruginosa* ATCC 15442 and *Staphylococcus aureus* ATCC 6538 were treated with Ag-NPs or AgNO<sub>3</sub> at 37 °C for 2 h. The cells were fixed with 2.5% glutaraldehyde for 24 h and post-fixed in 1% osmium tetroxide for 2 h at 25 °C, washed once in 0.1 M PBS and dehydrated by sequential transfer in a graded series of ethanol solutions. Fine sections were cut with diamond blades using ultramicrotome and double stained with uranyl acetate and lead citrate saturated and observed using the transmission electron microscope with EDX detector (FEI Tecnai-G2-20, 2006) available in microscopy center of Federal University of Minas Gerais.

**Conclusions**

The Ag-NPs affected severely *P. aeruginosa*, forming a condensation region in the center of the cell and a clear zone around. According to the EDX spectrum, the condensation region is composed mainly of silver, sulfur and phosphorus. However, these elements were not detected in the clear area. Damages to *S. aureus* were similar to those caused on *P. aeruginosa*, however, milder form. Damages caused by AgNO<sub>3</sub> were similar to those caused by Ag-NPs.



**FEMS-0903**

**New antimicrobial mechanisms**

**LIPID PEROXIDATION IS NOT RELATED TO THE ANTIMICROBIAL MECHANISM OF SILVER NANOPARTICLES AGAINST PSEUDOMONAS AERUGINOSA AND STAPHYLOCOCCUS AUREUS**

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**Background**

The antimicrobial activity of silver nanoparticles (Ag-NPs) may be related to the formation of reactive oxygen species (ROS). A common feature among the different ROS is the ability to cause some oxidative damage in cells. The lipids are the main targets during oxidative stress.

**Objectives**

The objective was to determine whether the Ag-NPs cause oxidative stress in the lipids of membranes of *Pseudomonas aeruginosa* or *Staphylococcus aureus*.

**Methods**

The Ag-NPs were synthesized according Monteiro et al. (2009), with some modifications. The lipid peroxidation analysis was performed according to Becerra et al. (2006), with some adjustments. Samples containing 50 µg/ml Ag-NPs or AgNO<sub>3</sub> were inoculated with 1.0 x 10<sup>8</sup> CFU/ml of *P. aeruginosa* or *S. aureus* and incubated at 37°C for 1 h. Then was added 1 ml of 35% trichloroacetic acid (w/v). After 20 min, the samples were treated with 1 ml of 0.5% thiobarbituric acid (w/v) and heated at 80°C for 30 min, then cooled and centrifuged. The absorbance was determined at 535 nm. The controls with water and 2,2'-azobios (2-methyl-propionamide) dihydrochloride (AAPH) were made. A standard curve of malondialdehyde was obtained by hydrolysis of 1'1'3'3'-tetraetoxipropano.

**Conclusions**

The concentration of malonaldehyde, a product of lipid peroxidation, in *P. aeruginosa* and *S. aureus* cells treated with Ag-NPs and AgNO<sub>3</sub> was not different ( $p > 0.05$ ) from cells treated with water, however, was lower from the cells treated with AAPH, a ROS generator. This indicates that lipid peroxidation is not involved in the antimicrobial activity of Ag-NPC and AgNO<sub>3</sub>.



## **FEMS-2156**

### **New antimicrobial mechanisms**

#### **ASSESSMENT OF E. COLI BIOFILM GROWTH ON DIFFERENTLY TERMINATED DIAMOND THIN FILMS**

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#### **Background**

Diamond is a material with interesting properties, such as chemical inertness, hardness, high transparency and good biocompatibility with eukaryotic cells. These qualities make this material very promising for various bio-medical applications such as biosensors, articular implant coatings, tissue-engineering and regenerative medicine. Several published works also indicate that diamond thin films, due to their tailored surface properties, could possess anti-adhesive or even antimicrobial properties.

#### **Objectives**

The objectives of our work is to develop and standardize the methods for diamond thin film deposition on glass substrate, to develop and optimize the methods for testing of antibacterial properties of diamond surfaces, to assess these properties and to compare the influence of diamond surface terminations on their possible antibacterial character.

#### **Methods**

We used diamond films CVD deposited by microwave plasma CVD process. *Escherichia coli* was chosen as the model bacterium. Bacterial biofilm was cultivated on diamond-coated glass in CDC biofilm reactor. Fluorescence microscopy and ATP assay was used to quantify bacterial growth on diamond surfaces.

#### **Conclusions**

We managed to deposit diamond thin films of standard properties on glass over large areas (1x3"). We successfully developed the continuous cultivation method for biofilm growth and testing of antibacterial properties of diamond thin films under different conditions. We compared the antibacterial potential of diamond films with different surface terminations (H, O and F).



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**FEMS-1662**

**New antimicrobial mechanisms**

**SUSCEPTIBILITY OF FOOD CONTAMINATING MICROBIAL COMMUNITIES TO ATMOSPHERIC COLD PLASMA AND IMPLICATIONS FOR RESISTANCE-PROOF ANTI-MICROBIAL TREATMENT**

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**Background**

Atmospheric cold plasma (ACP) has received increasing attention as a novel anti-microbial treatment, which may be less prone to resistances. Containing ions, free electrons, UV light and reactive species, plasma can cause damage to microbial cell walls and/or intra-cellular components.

**Objectives**

To determine the anti-microbial efficacy of ACP on food-contaminating microorganisms and their ability to develop resistance to plasma treatment.

**Methods**

The inactivation of a range of common microbial food contaminants using di-electric barrier discharge ACP was investigated and cultures of *S.aureus* and *E.coli* were subjected to repeated sub-lethal plasma treatment. The background microflora of plasma-treated meat products was analysed over 2-4 week storage and community profiling was performed using PCR-amplification and denaturing gradient gel electrophoresis (DGGE) of 16s RNA.

**Conclusions**

Microorganisms displayed varying susceptibility to ACP, with *C.jejuni* and *B.thermosphacta* showing rapid inactivation whereas *E.coli*, *L.monocytogenes* and *S.aureus* required longer treatment times. Repeated selection of sub-lethally treated colonies did not increase resistance in short-term adaptation studies. The background microflora of meat products was reduced up to 1log over 2-4 week storage following treatment and PCR-DGGE revealed differences in plasma tolerance between microbial populations. In an era of growing anti-microbial resistances, resistance-proof alternative treatments are gaining significance. ACP has shown anti-microbial effects on a variety of microorganisms and acts through a range of physico-chemical effects that may prevent the development of resistances. Such novel anti-microbial strategies have implications not only in the food industry but also the medical sector but will require long-term adaptation studies to ensure resistance-proof.



**FEMS-0966**

**New antimicrobial mechanisms**

### **SELF DECONTAMINATING PHOTOCATALYTIC TEXTILES**

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#### **Background**

Healthcare textiles are known to be able to transfer pathogens.

#### **Objectives**

To provide new antimicrobial textiles, we functionalized decontaminating photocatalytic textiles by the innovative LBL (Layer-by-Layer) technique. Photocatalysis deals with the activation of TiO<sub>2</sub> semiconductor particles by UVA, which produces highly Reactive Oxygen Species responsible for the microorganisms inactivation.

#### **Methods**

The LBL coating is based on successive application of positively charged layers and negatively charged layers. To improve the adhesion of the first layer on the fibers, the textile was first submitted to a hydrolysis using KOH solution. Negatively-charged TiO<sub>2</sub> particles with a polyethylenimine (PEI) polycation layer were alternatively spraying onto textiles. Another functionalization was also performed by adding an interfacial layer of PEI before building bilayers of alternatively positively-charged TiO<sub>2</sub> particles with a poly(styrene sulfonate) (PSS) polyanion. These sequence were repeated 1-5-10 times until (PEI/TiO<sub>2</sub>)<sub>n</sub> or PEI/(PSS/TiO<sub>2</sub>)<sub>n</sub> bilayers were achieved. The antibacterial properties of functionalized textiles against *E.coli* were determined after 15-30-60min of solar light radiation (226W/m<sup>2</sup> of visible light).

#### **Conclusions**

Whereas no antibacterial effect was observed for the textiles pretreated by KOH and kept in the dark, we respectively obtained a 2log reduction for the textiles functionalized with only one layer of PEI and a 6log reduction with addition of a single layer of TiO<sub>2</sub> (PEI/TiO<sub>2</sub>)<sub>1</sub>. For the PEI/(PSS/TiO<sub>2</sub>)<sub>n</sub> textiles, antibacterial activity is function to solar light duration and also depends of the number of bilayers. The best antibacterial activity (4log reductions) was obtained for PEI/(PSS/TiO<sub>2</sub>)<sub>10</sub> under 60min of solar light radiation.

## **FEMS-0730**

### **New antimicrobial mechanisms**

#### **DETERMINING GROWTH INHIBITION MECHANISMS OF THE ANTIMICROBIAL AGENT FREE NITROUS ACID (FNA) FROM THE TRANSCRIPTOME RESPONSE OF PSEUDOMONAS AERUGINOSA PAO1**

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### **Background**

Free nitrous acid (FNA) has recently been demonstrated as an antimicrobial agent to a range of microorganisms. However, the antimicrobial mechanism of FNA is largely unknown.

### **Objectives**

Consequently, we aim to gain a systematic understanding of the bacteriostatic/bactericidal effects of FNA. This was examined on *Pseudomonas aeruginosa* PAO1, an organism with intrinsic resistance to various antibiotics and disinfectants.

### **Methods**

*P. aeruginosa* was grown under anaerobic denitrifying conditions and when FNA was added (0.05 mg N/L) growth temporarily stopped. From cultures either exposed or not exposed to added FNA, RNA was extracted and transcripts were detected by HiSeq-Illumina sequencing. Transcripts were quantified and those exhibiting  $\geq 2.5$  or  $\leq 2.5$  fold abundance change were designated as differentially expressed in response to FNA.

### **Conclusions**

In response to FNA 177 genes showed increased transcription while 471 genes exhibited decreased transcript levels. Genes coding ribosome proteins showed significantly decreased transcript levels, implicating that protein biosynthesis was severely inhibited by FNA. Respiration was likely inhibited, as lowered substrate utilization coincided with decreased transcript levels of genes coding for denitrification. In contrast, genes encoding nitric oxide reductase were up regulated, which possibly functioned to remove toxic NO derived from the added nitrite. In the response, genes of the tricarboxylic acid cycle were less expressed, while enzymes of the glyoxylate shunt had increased levels. Evidently the cells would generate less reducing power through these cycles. This first investigation elucidating the

mechanisms of FNA effect on *P. aeruginosa*, contributes underpinning knowledge for potential application of this novel antimicrobial agent.

**FEMS-1375**

**New antimicrobial mechanisms**

## **ELUCIDATION OF THE MODE OF ACTION OF A NEW ANTI-PSEUDOMONAL COMPOUND**

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### **Background**

*Pseudomonas aeruginosa* is a gram-negative pathogen that causes severe infections in immunocompromised patients and is responsible for 10 – 15% of the hospital-acquired infections worldwide. Due to the emergence of multidrug-resistant *P. aeruginosa* strains, there is an urgent need for the development of new antibiotics with activity against *P. aeruginosa*. We recently identified a new antibacterial compound, SPI003, which inhibits growth of various gram-positive and gram-negative pathogens, including *P. aeruginosa*.

### **Objectives**

In this study, we sought to unravel the mode of action of SPI003, using *P. aeruginosa* as a model organism.

### **Methods**

In order to unravel the mode of action of SPI003, different approaches were used. First, a library of *P. aeruginosa* mutants was screened to identify mutants that are sensitive or resistant to SPI003. Second, spontaneous resistant mutants were generated by plating *P. aeruginosa* cells on agar plates supplemented with SPI003 at a final concentration of 5 x MIC. Third, a transcriptome analysis was carried out on exponentially-growing cells of *P. aeruginosa* treated with the compound. Finally, the membrane permeability of *P. aeruginosa* was examined after exposure to SPI003 using established assays.

### **Conclusions**

Our study shows that SPI003 exhibits a fast membrane-damaging activity, which is likely to be the primary cause of its antibacterial activity.

**FEMS-1856**

**New antimicrobial mechanisms**

## **INVESTIGATION OF ATMOSPHERIC COLD PLASMA AGAINST ESCHERICHIA COLI MUTANTS**

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### **Background**

Atmospheric cold plasma (ACP) is effective against a wide range of pathogenic microorganisms with promising application in food and medical industry. Generation of reactive oxygen species (ROS) plays a crucial role when air/oxygen containing gases are used. As a mixed group of oxidative reactive species generated by ACP, there are limited publications about the cellular regulation system under plasma pressure.

### **Objectives**

To investigate the effect of the plasma stress on the microbial inactivation, intracellular reactive oxygen species and genetic regulation.

### **Methods**

*Escherichia coli* BW 25113 and its isogenic mutants in *soxR*, *soxS*, *oxyR*, *rpoS* and *dnaK* genes were suspended in phosphate buffered saline (PBS) at  $10^8$  CFU/ml. Samples were then treated with high voltage plasma (80 kV<sub>RMS</sub>) in a sealed package for 1, 3 and 5 min following 0, 1 and 24 h post-treatment storage. Intracellular ROS were measured using fluorescence spectrophotometer (Biotek synergy, 480/530nm) with the probe 2', 7'- dichlorofluorescein diacetate immediately after storage.

### **Conclusions**

Without post-treatment storage time, the absence of RpoS and OxyR led to higher reduction after treatment. However, DnaK showed its repairing function correlated to cell survival with 24 h, while it has no effect on reducing intracellular ROS level. SoxS became important with both 1 and 24 h storage, which is not observed in SoxR. It implied their different characteristics as subunits. Overall, the cell response against plasma generated oxidative stress could be divided to short term and long term, which are dominated by oxidative stress deletion genes and damage repairing genes.





**FEMS-1772**

**New antimicrobial mechanisms**

## **NEW ENVIRONMENTAL-FRIENDLY COMPOUNDS TO COMBAT CITRUS CANKER**

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### **Background**

The culture of citrus fruits is constantly under the threat of plant diseases. One of them is citrus canker, which is caused by the bacterial plant pathogen *Xanthomonas citri* subsp. *citri* (Xac). Current modes of prevention of Xac infection and spreading include spraying trees with copper containing bactericides and eradication of infected and healthy trees in a certain radius around. Despite these efforts, about 300 Million tons of fruit per year were lost during the last decade.

### **Objectives**

In addition to being inefficient, copper containing bactericides negatively impact soil quality since copper accumulates in the environment. Therefore, more sustainable ways to combat citrus canker are urgently needed. Recently, a class of plant-derived semi-synthetic compounds, the alkyl gallates, has shown promising activity against Xac [1] and the bacterial cell division protein FtsZ has been found to be their target (unpublished).

### **Methods**

Here, we present *in vitro* results on the activity of the alkyl gallates against FtsZ from different bacteria. We also discuss the ideal alkyl chain length of a lead gallate candidate as a platform for chemical synthesis of new compounds with enhanced antibacterial activity.

### **Conclusions**

Our initial results show that the development of novel environmental-friendly antibacterials to combat Xac is feasible. The efficacy of these compounds in the field will be evaluated and their toxicity to plants and animals will be determined. We expect that the compounds can be used as a sustainable alternative to prevent citrus canker and they will contribute to control the spread of Xac in citrus plants.

[1] Silva I.C., et al., J Bacteriol 2013,195(1):85-94.

**FEMS-2414**

**New antimicrobial mechanisms**

## **MECHANISMS OF ANTIBACTERIAL ACTION OF CATIONIC PEPTIDE WARNERIN**

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### **Background**

Rapid development of antibiotic resistance of bacteria necessitates the search for new natural compounds inhibiting the growth and functioning of pathogenic microflora.

### **Objectives**

To study the antibacterial mechanisms of warnerin, a new peptide from lantibiotic family that demonstrate the sequence of events causing the death of attacked bacterial cells.

### **Methods**

Outcomes of warnerin attack of *Staphylococcus epidermidis* 33 GISK (Moscow) were analyzed using the combination of different methods such as polarography (respiration), spectrography (intracellular potassium loss), fluorescence assay of membrane potential dynamics and hydroxyl radicals' formation, electron and atomic-force microscopy of cell structure damages

### **Conclusions**

Peptide introduction into the bacterial incubation medium results in the increase in their oxygen consumption followed by rapid cellular release of potassium ions and sharp decline of intracellular ATP concentration. Apparently, the disturbance of ATP-linked electron transport along the respiratory chain favors their accumulation on iron-sulfur centres. This results in increase of free Fe<sup>2+</sup> ions and as a consequence, to the elevation of hydroxyl radical concentrations. Peptide inhibition of ATP formation is accompanied by marked proton accumulation on the outer side of bacterial membrane, particularly in its adjacent layers of peptidoglycane. This facilitates the autolytic hydrolase extrusion from the binding sites and to uncontrolled total bacteriolysis that is revealed by microscopic data. Results of investigation evidence for the pluripotent antibacterial action of warnerin that point to expressed prospects for its practical use.

**The work was supported by RFBR grant No. 14-04-00687\_a.**

## FEMS-1702

### New antimicrobial mechanisms

#### FUNCTIONALIZATION OF PROTOPORPHYRIN IX WITH ANTIMICROBIAL PEPTIDES TO ENHANCE PHOTOANTIMICROBIAL CHEMOTHERAPY (PACT).

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#### Background

Photodynamic antimicrobial chemotherapy (PACT) combines activities of light, photosensitizing compound (PS) and oxygen, to eradicate microorganisms through the oxidation- related mechanisms. To obtain more specific photodynamic action, PS can be functionalized with various types of adducts. Presented approach combines activity of a photosensitizer and antimicrobial peptides (AMPs).

#### Objectives

Protoporphyrin IX (PPIX) covalently conjugated with antimicrobial peptides: PEX or CAM, was analyzed for its spectral properties and antimicrobial action. Bacterial strains used in the study were *Staphylococcus aureus* Newman (MSSA) and COL (MRSA).

#### Methods

Peptides were synthesized using the methodology Fmoc/tBu on polystyrene resin and purified by HPLC. MIC values were determined by the broth dilution method in BHI medium in the darkness. Absorbance spectra screening was performed using the EnVision™ microplate reader (PerkinElmer) within 350-700 nm range. PACT was conducted using the LED lamp (SecureMedia, Poland) emitting incoherent red light ( $\lambda_{\max}$  627 nm). Viability of bacteria was calculated counting colony forming units (CFU).

#### Conclusions

Combination of pure compounds showed no influence on PPIX spectral properties and AMPs activity by means of MIC values and showed synergistic effect of PPIX and CAM in PACT experiments. PS-AMP conjugates reveal weaker antimicrobial activity by means of MIC values, as compared to pure peptides. Conjugation of peptides to PPIX changed its spectral properties, visualized by hypsochromic shift of absorbance spectra, hyperchromic effect in Soret band and hypochromic effect in Q bands. These observation implied weaker/no photodynamic activity induced by red light, which was confirmed experimentally, however indicated efficient activity induced by blue light.

**FEMS-0978**

**New antimicrobial mechanisms**

**SILVER NANOPARTICLES INHIBIT MOTILITY, PYOVERDINE PRODUCTION AND BIOFILM FORMATION BY PSEUDOMONAS AERUGINOSA**

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**Background**

Antimicrobial efficacy of silver nanoparticles (AgNPs) was proved and broad applications were proposed so far. This study shows the potential mechanism of AgNPs action towards opportunistic pathogen *Pseudomonas aeruginosa*.

**Objectives**

Study of the AgNPs effect on selected *P. aeruginosa* virulence factors.

**Methods**

*P. aeruginosa* PAO1 was used in this study. Silver nanoparticles were obtained from Nano-Tech (Warsaw, Poland). Minimal inhibitory concentration of silver nanoparticles that inhibit biofilm formation (MBIC) was estimated by standard two-fold dilution method. Swimming motility was examined on 0.3% agar plates and swarming motility on 0.6% agar plates supplemented with 0.05–0.5xMBIC AgNPs. Twitching motility across the glass surface was analysed after staining with crystal violet. Pyoverdine production was estimated in 48-hour PAO1 biofilms grown in microtitre plates. Biofilm formation was studied after crystal violet staining and cell survival was determined in Live/Dead Assay (Promega). Statistical analysis of the results in comparison to control sample without AgNPs was performed.

**Conclusions**

Strong antibacterial effect of AgNPs was observed. MBIC was 4 microgram mL<sup>-1</sup>. There was significant decrease from 22% to 40% in swimming and from 22% to 29% in swarming motility in samples containing 0.1–0.5xMBIC of AgNPs in comparison to *P. aeruginosa* PAO1 incubated without nanoparticles. No influence of AgNPs on twitching motility was observed. Pyoverdine production was significantly inhibited by AgNPs in concentration of 0.5 MBIC. The inhibition of biofilm formation by 44% was also observed. **Hence, it was shown that** silver nanoparticles affect several virulence factors of *P. aeruginosa* including swarming, swimming motility, pyoverdine production and biofilm formation.

## **FEMS-1986**

### **New antimicrobial mechanisms**

#### **ANTIBIOTIC ACTIVITY OF MICROBIAL STRAINS ISOLATED FROM MYTILUS EDULIS, KIEL FJORD, BALTIC SEA**

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### **Background**

Microorganisms associated with eukaryotic hosts are involved in various physiological functions, including chemical defense and immune responses. Production of bioactive molecules such as antibiotics and antifouling substances by associated bacteria, is one of the mechanisms to protect the host against pathogens, predation and surface colonization.

### **Objectives**

In this study, extracts from 10 bacterial strains isolated from the blue mussel, *Mytilus edulis* (Kiel Fjord, Baltic Sea, Germany), were screened for their antimicrobial activity against two panels of test microorganisms: a standard panel (*Escherchia coli*, *Bacillus subtilis* and *Candida albicans*) and environmental panel composed of strains isolated from the mussels (*Pseudomonas veronii*, *Bacillus pumilus* and *Shewanella baltica*).

### **Methods**

To identify isolated strains, PCR amplification of 16S rRNA was carried out and bioactivity tests were modified according to Schneemann et al. (2010) to evaluate the interaction of isolated strains with the test microorganisms.

### **Conclusions**

Inhibiting effects varied considerably among the test microorganisms, with *P. veronii* showing the most pronounced antibacterial activity against both the standard and the environmental panels. Moreover, the Gram-positive bacteria, and particularly *B. pumilus*, were generally more susceptible than Gram-negative strains, whereas no strain was found to inhibit growth of *E. coli*. These findings suggest that bacteria associated with the blue mussels may be involved in preventing pathogen invasions and that these hosts represent a promising source for the isolation of

antibiotic-active bacteria.



FEMS-1088

New antimicrobial mechanisms

## CHALCONE AND ITS HYDROXYLATED ANALOGUES: SYNTHESIS, ANTI-CRYPTOCOCCUS AND CYTOTOXIC ACTIVITIES

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**Background** Cryptococcosis is a potentially fatal systemic mycosis, which is caused by *Cryptococcus neoformans*(Cn) and *C. gattii* (Cg). This disease is an opportunistic infection for AIDS. *Cryptococcus* is able to cause three types of infections, cutaneous cryptococcosis, pulmonar cryptococcosis and cryptococcal meningitis. The treatment recommended is the intravenous amphotericin B with flucytosine administered orally. This drug combination causes severe side effects and yeast-resistance, encouraging the search for innovative anti-*Cryptococcus* agents. **Objectives** The objectives of this work were synthesis of chalcone and hydroxychalcones, determination of antifungal activity against Cn and Cg strains, evaluation of cytotoxicity against mammalian cell lines, and evaluation of the inhibitory cell adhesion activity of Cn onto pneumocytes. **Methods** The chalcone and hydroxychalcones were synthesized by Claisen-Schmidt condensations. The anti-*Cryptococcus* activity was evaluated by microdilution test performed in 96-well plates, using CLSI-M27-A2 document. This assay demonstrated the values of Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) to five strains; Cn-ATCC90012, and four clinical isolates CnS, CnI, CnR and Cg118. The cytotoxicity was evaluated by MTT colorimetric assay, using five mamalian cell lines, A549, HepG2, MRC-5, U87-MG and NOK. **Conclusions** The hydroxychalcones exhibited potent antifungal activity, demonstrating MIC and MFC values ranging from 1.95 to 15.6µg/mL, which were related to high inhibition of Cn adhesion onto pneumocytes. In MTT assay, at 100µg/mL, these compounds were not toxic for all cell lines. We conclude that hydroxychalcones are antifungal hits, with relative selectivity for yeast, and their mode of action is related to inhibition of yeast adhesion onto host-cells.

**FEMS-1859**

**New antimicrobial mechanisms**

## **IN VIVO CLUSTER FORMATION OF NISIN AND LIPID II IS CORRELATED WITH MEMBRANE DEPOLARIZATION**

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### **Background**

Nisin and related lantibiotics can kill bacteria in two ways: by pore formation, which leads to membrane depolarization, or by sequestering Lipid II, which interferes with cell wall synthesis. Literature shows that during sequestration, nisin and Lipid II cluster into non-physiological domains, which are visible with fluorescence microscopy. Recently we observed that a reported 'non-pore' forming nisin mutant clusters LipidII, but also depolarizes the membrane (Lages *et al.*, 2013, Environm. Microbiol. 15, 3272).

### **Objectives**

To further investigate the relation between LipidII clustering and membrane depolarization.

### **Methods**

Microscopy, fluorescent assays to detect membrane integrity.

### **Conclusions**

We used *in vivo* assays to investigate how and when nisin forms pores, depolarizes the membrane, and segregates Lipid II into domains. By comparing the activity of pore-forming and pore-deficient mutants of nisin we show that clustering of nisin and Lipid II *only* occurs when (i) membrane integrity is reduced by pore formation and (ii) when the membrane is completely depolarized. Furthermore, pore-forming variants of nisin were also able to kill L-forms – which grow without a cell wall – whereas non-pore forming lantibiotics only kill bacteria with a cell wall. Our results have important implications for the mechanism-of-action of nisin. Because cluster formation never occurred with non-pore-forming lantibiotics, we suggest that lantibiotics either kill by pore-formation, or by occlusion of Lipid II for incorporation into the cell wall.

**FEMS-0562**

**New antimicrobials for resistant organisms**

## **ENHANCEMENT OF STAPHYLOCOCCUS AUREUS MRSA ANTIBIOTIC SUSCEPTIBILITY USING PHENOLIC COMPOUNDS FROM PLANTS**

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### **Background**

Combinatory effect between conventional antimicrobial drugs and phytochemical products can be a new strategy to increase effectiveness of antibiotics.

### **Objectives**

We aimed to verify synergism between essential oils compounds from plants and antimicrobial drugs for methicillin-resistant *Staphylococcus aureus* (MRSA).

### **Methods**

Five essential oils compounds (citronellol, geraniol, eugenol, terpineol and cinnamaldehide) were tested against 10 strains of *S. aureus* MRSA. Mueller-Hinton agar (MHA) plus 0.5% Tween 80 was mixed with each compound ( $\frac{1}{4}$  of Minimal Inhibitory Concentration (MIC<sub>90%</sub>)). Nine antimicrobial drugs discs (oxacillin (1 µg), gentamicin (10 µg), erythromycin (15 µg), sulfazotrim (25 µg), vancomycin (30 µg), penicillin G (10 U) levofloxacin (5 µg), tetracycline (30 µg) and linezolid (30 µg)) were laid on culture medium inoculated with *S. aureus*. Assays were performed in duplicate. Mann-Whitney test was used as statistical. Time kill curves were performed between EOC and AD for positive cases. Treatments were incubated with 10<sup>5</sup> CFU/mL of MRSA strain in MH broth. Media without EOC and/or AD was used as control. Aliquots were removed at different time intervals between 0 and 24h. Viable colonies were counted after incubation (24 h/ 37°C) in order to obtain CFU/mL.

### **Conclusions**

The present study showed a promising way of treatment for diseases caused by MRSA as well as against other bacterial species, because time kill curves for EOC and AD demonstrated additive activity. Thereby, these compounds can be used in combination with conventional antimicrobials.



**FEMS-2674**

**New antimicrobials for resistant organisms**

**SENSITIVITY OF MDR ACINETOBACTER BAUMANNII WOUND ISOLATES TO RUMEX SANGUINEUS, RUMEX CRISPUS AND URTICA DIOICA EXTRACTS**

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**Background**

*Acinetobacter baumannii* is a pleomorphic, non-motile bacterium and etiological agent of various infections in immunocompromised patients. It has been characterized as a novel, drug resistant and rapidly emerging clinical pathogen with increasing prevalence. Regarding recent multi-drug resistant *A. baumannii* strains emergence, it is urgent to examine alternative antimicrobial agents, including various plant extracts.

**Objectives**

The aim of this study was to determine *in vitro* antimicrobial activity of *Rumex crispus*, *Rumex sanguineus* and *Urtica dioica* extracts against multi-drug resistant *A. baumannii* wound isolates.

**Methods**

The broth microdilution assay was used to determine minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) of 8 plant extracts obtained from different parts of the examined plants. For antibacterial activity testing 20 multiple resistant *A. baumannii* wound isolates and 2 reference strains (ATCC BAA-747 and ATCC 19606) were used.

**Conclusions**

*Urtica dioica* possessed no effect against *A. baumannii*, while *Rumex sanguineus* and *Rumex crispus* extracts showed significant antibacterial activity against all examined isolates. Bacteriostatic effect was obtained with concentrations 1 - 4 mg mL<sup>-1</sup> with median value 2 mg mL<sup>-1</sup>, while bactericidal activity was achieved with a slightly higher concentrations of *Rumex* extracts (1 to 8 mg mL<sup>-1</sup> with median 4 mg mL<sup>-1</sup>). The obtained result confirmed earlier detected antibacterial effect of *R. crispus* extracts against *A. baumannii*, while anti-*A. baumannii* activity of *R. sanguineus* extracts was detected for the first time. Accordingly, the plants from the genus *Rumex* are valuable source of bioactive compounds against this bacterium and antimicrobial activity of dominant extract components should be further examined.

## FEMS-2600

### New antimicrobials for resistant organisms

#### CHARACTERIZING THE STAPHYLOCOCCUS AUREUS ANTI-VIRULENCE CANDIDATE SOLONAMIDE B AND ITS LACTAM ANALOGUES

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#### Background

*Staphylococcus aureus* infections are becoming increasingly difficult to treat due to antibiotic resistance. Of particular concern are the community-associated methicillin-resistant *S. aureus* (CA-MRSA) strains such as USA300. Virulence of CA-MRSA is regulated by the *agr* quorum-sensing system, which in response to *agr*-encoded autoinducing peptides confines exotoxin production to the stationary growth phase with concomitant repression of surface-expressed adhesins. In need of novel treatment options, anti-virulence therapy, targeting virulence factors rather than viability, has been proposed. Solonamide B was recently isolated from a marine bacterium as a putative anti-virulence compound that markedly reduced expression of the toxins  $\alpha$ -hemolysin and the phenol-soluble modulins.

#### Objectives

To further strengthen the anti-virulence candidacy of Solonamide B and synthesized analogues.

#### Methods

Chemically synthesized solonamides and lactam analogues were validated for interference with *agr* virulence gene regulation, both quantitatively and qualitatively, using reporter fusion strains. The compounds' influence on biofilm formation and fibronectin binding of *S. aureus* were assessed as examples of complications related to *agr* negative strains; and lastly, toxicity of the compounds and the immune modulating capacity of solonamide-treated *S. aureus* were assessed on PBMCs, T-cell populations and DCs via flow cytometry.

#### Conclusions

We find that structural differences between solonamide analogues interfere differentially with *agr*, and that they do not display toxicity or influence immune cell activity and integrity. Importantly, our results also dismiss the major concern that application of compounds mimicking an *agr*-negative state may lead to adverse

interactions with host factors and immune cells.

**FEMS-1524**

**New antimicrobials for resistant organisms**

**ANTIMICROBIAL EFFECT OF LIPOSOMES MODIFIED BY LYSOSOMAL MEMBRANE PROTEINS EXTRACTED FROM OVEREXPRESSED HSP70 IN *S. CEREVISIAE***

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**Background**

Preparation of liposome surface modified by various materials such as RNA, DNA or Proteins has been developed for several decades.

**Objectives**

We would like to investigate targeting ability of lysosomal membrane proteins related to killing bacteria through modifying the liposome surface to check the possibility of as antimicrobial agents whereby we tried to find specific membrane proteins.

**Methods**

Firstly, we used mixtures of lipid, cholesterol and lysosomal membrane protein isolated from lysosomes extracted from *S. cerevisiae* treated by H<sub>2</sub>O<sub>2</sub> and NH<sub>4</sub>Cl to modify liposome surface for antimicrobial activity after making liposomes prepared with hydration method. Liposomes were modified by lysosomal membrane proteins with two different methods in order to confirm the targeting ability.

**Conclusions**

The results showed that the antimicrobial activity was not shown without modification of liposome surface but it was better cell mortality in type 1 than type 2 method. Based on these results, we analyzed whole lysosomal membrane proteins by 2-DE assay to find specific proteins associated with antimicrobial activity and constructed recombinant *S. cerevisiae*. And then, we found the genes (HSP70) related to antimicrobial activity and prepared liposomes modified by lysosomal membrane proteins extracted from lysosomes isolated from recombinant *S. cerevisiae* tagged with GFP. The result showed that the antimicrobial activity of liposomes modified with Type1 method was better than control. Therefore, lysosomal membrane proteins for targeting bacteria will play an important a role.

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**FEMS-2987**

**New antimicrobials for resistant organisms**

**DOUBLE LEADING: LANTIBIOTIC - MICROCIN HYBRIDS**

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**Background**

Lantibiotics and microcins form different groups of posttranslationally modified bacterial peptides mainly produced by Gram-positive and Gram-negative bacteria, respectively. Most of these peptides exhibit a potent antimicrobial activity, more active even than some antibiotics. Maturation of a peptide begins with a propeptide which is guided throughout specific modification events due to leader peptide (an amino acid sequence recognized by particular modification biomodule). The leader peptide is proteolitically removed in the last steps of peptide modification making the modified peptide active. Posttranslational modifications endorse lantibiotics and microcins with high target-specific activities and stability against proteolysis.

**Objectives**

To design plug-and-play biomodules for production of novel peptides containing both, lantibiotic- and microcin-specific posttranslational modifications.

**Methods**

We used a synthetic biology approach to design biomodules. We utilized peptide modification systems cloned under different controlled promoters to assure gradual and temporal activity of designed biomodules. Codon optimized synthetic genes of designed model peptides to be modified were employed together with modification machineries.

**Conclusions**

This is a unique case where posttranslational modifications from two different peptide classes are fused together into a single peptide chain *in vivo*. Here, we also show the possibilities of employing chimeric leader peptides for substrate recognition and procession by particular posttranslational modification machineries.

**FEMS-2060**

**New antimicrobials for resistant organisms**

**SILVER NANOPARTICLES WITH ANTIMICROBIAL ACTIVITY FOR BACTERIA  
GRAM POSITIVE, NEGATIVE AND YEAST**

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**Background**

Silver and its compounds have strong antimicrobial activities for bacteria, fungus, and viruses. Recent advances in researches made on metal nanoparticles appear to revive the use of silver nanoparticles (SNPs) for antimicrobial applications. It has been shown that SNPs prepared with a variety of synthetic methods have effective antimicrobial activity.

**Objectives**

In this work, silver nanoparticles (Ag NP) with 5% silver chloride (NanoRa<sup>2</sup>), were used to evaluate the antimicrobial activity against various bacteria Gram-positive, Gram-negative and yeasts.

**Methods**

Silver nanoparticles (NanoRa<sup>2</sup>) were synthesized with silver chloride to 5%, by the Sol-Gel method. Silver nanoparticles were characterized by SEM (Scanning Electron Microscope). Silver nanoparticles (NanoRa<sup>2</sup>) were tested for different Gram-positive bacteria as *Enterococcus faecalis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, and Gram-negative bacteria such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis* and *vulgaris*, as well as the yeast *Candida albicans*. The antibacterial activity of the silver nanoparticles was evaluated following the guidelines for the Kirby-Bauer diffusion method according to the CSLI.

**Conclusions**

Nanoparticles have a particle size of 100nm. The spectrophotometric profile showed characteristic IR peaks, links titanium dioxide that have the NanoRa<sup>2</sup>. The silver nanoparticles were able to inhibit the growth of Gram-positive, Gram-negative and yeasts from 10 ppm and 50 ppm. In the case of *Staphylococcus aureus* methicillin resistant as well as *Candida albicans*, the silver nanoparticles at a concentration of 50 ppm, larger zones of inhibition were showed.

## **FEMS-1176**

### **New antimicrobials for resistant organisms**

#### **P113-DERIVED PEPTIDES AS NOVEL INHIBITORS FOR DRUG-RESISTANT CANDIDA SPP.**

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#### **Background**

*Candida* spp., major opportunistic pathogens, infects immunocompromised people and is responsible for life-threatening infections. The individuals commonly affected include those with oral candidiasis of HIV-positive patients, cancer patients undergoing radiotherapy, and xerostoma patients. *Candida* infections may further result in severe systemic infection and convert multiple organ failure (1). Moreover, *Candida* spp. can develop resistance to multiple antifungal drugs by over-expression of drug resistance-related targets.

#### **Objectives**

In this study, we developed a patented anti-fungal peptide P-113 to overcome the emerging issue of drug-resistance in *Candida* infections. We also modified P-113 by tandemly repeating sequences to improve the candidacidal efficiency.

#### **Methods**

The P-113, derived from human histatin-5 protein, retains a full antibacterial activity and exhibits a wide spectrum of activity in vitro against both bacteria and fungi (2). Several drug- (azoles-) resistant clinical strains including *C. albicans*, *C. krusei*, *C. glabrata*, and *C. tropicalis* were isolated from oral candidiasis patients. We investigated the susceptibility of these drug-resistant strains to P-113 by candidacidal assay (3).

#### **Conclusions**

The results showed that P-113 was able to kill these drug-resistant strains, suggesting a promising candidacidal activity of P-113 toward drug-resistant *Candida* spp. To improve the candidacidal activity, we designed P-113-derived dimeric and trimeric peptides which contain 2- and 3-time tandem repeats. The two tandem repeats improved the candidacidal potency of the peptides, and the dimer and trimer

have  $IC_{50}$  values against *C. albicans* of 0.5 and 0.4  $\mu M$ , respectively, compared with the monomer P-113 ( $IC_{50} = 2.3 \mu M$ ). P-113 and its derivatives show a potential for the development of novel therapeutics against *Candida* infections.

**FEMS-1098**

**New antimicrobials for resistant organisms**

**SYNERGISM OF FLORFENICOL IN COMBINATION WITH ANALOG  
THIAMPHENICOL AGAINST STAPHYLOCOCCUS HYICUS, STREPTOCOCCUS  
SUIS, AND PASTEURELLA MULTOCIDA**

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**Background**

Florfenicol (FF) and thiamphenicol (TAP) are amphenicol antibiotics commonly used in veterinary and aquiculture practice with broad-spectrum antibacterial activity. Previous study found that FF and TAP exhibited synergistic effect against *Staphylococcus aureus* (including methicillin-resistant) clinical isolated strains.

**Objectives**

To further investigate the spectrum of synergistic activities of FF and TAP combination.

**Methods**

The antibacterial activities of FF and TAP combination against a total of 69 strains of *Salmonella enterica*, *Pasteurella multocida*, *Pseudomonas aeruginosa*, *Riemerella anatipestifer*, *Streptococcus suis*, and *S. hyicus* were evaluated by checkerboard assay and time-kill study. For protective effects of combination therapy on chicken, FF and TAP alone or in combination were administered intramuscularly 30 mins prior to the intramuscular infection of *P. multocida*.

**Conclusions**

The checkerboard assay showed additive effect ( $0.5 \leq$  fractional inhibitory concentration (FIC) index  $\leq 1$ ) of FF/TAP combination in 75% of *P. multocida*, 53% of *S. suis*, 50% of *S. hyicus*, 33% of *P. aeruginosa* and 11% of *S. enterica* isolates that also include FF and TAP-resistant strains. In addition, 8 strains of *P. multocida*, *S. suis*, and *S. hyicus* were further demonstrated in the time-kill study confirming synergism between these two drugs. It also appeared that the combination enhanced the bacteriostatic effect to bactericidal on the 3 bacteria. Most notably, the *P. multocida*-challenged chickens were effectively protected by FF /TAP combination at lower dosages. Present study demonstrated the potentiality of a novel combination regimen of the same class of antibiotics against *P. multocida* infections.

## **FEMS-2460**

### **New antimicrobials for resistant organisms**

#### **DISCOVERY OF NEW ANTIMICROBIALS FROM ACTINOALLOMURUS**

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#### **Background**

These past few years have been changing dramatically the landscape of infectious diseases, showing that investment on drug discovery should not be disregarded as a steady emergence and spread of antibiotic resistance keeps occurring and giving origin to more multi-drug resistant strains. One possible source to enhance the antibacterial arsenal is to look on what Nature has been using to solve the very same problem. With the myriad of species waiting to be scrutinized, it is wise to give priority to those less exploited but phylogenetically related to known producers.

#### **Objectives**

This project is part of a screening program designed to assess the metabolic potential of the recently described genus *Actinoallomurus*, and to deliver new molecular structures.

#### **Methods**

The program includes small-scale fermentations followed by in vitro and in vivo bioactivity tests on fermentation broths extracts. Positive samples are then subjected to a combination of bioassays and chemical analysis (HPLC, MS and NMR and a query within ABL (a proprietary antibacterial database) to determine the novelty of active compounds and discard those already known.

#### **Conclusions**

Our study shows *Actinoallomurus* carries the biosynthetic machinery to create a vast range of different active molecules. The dereplication process of 200 *Actinoallomurus* microbial extracts revealed the potential to produce a broad range of chemical classes, from lantipeptides to new hyperhalogenated angucyclines or aromatic polyethers. The diversity of the clusters of secondary metabolism found in the sequenced genome of an *Actinoallomurus*, seems to corroborate the theory that this genus may become an important producer of new active molecules.

**FEMS-0699**

**New antimicrobials for resistant organisms**

**OPSONOPHAGOCYTOSIS RESPONSE IN MICE IMMUNIZED WITH CIH RECOMBINANT PROTEIN FROM STAPHYLOCOCCUS AUREUS**

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**Background**

The human pathogen *Staphylococcus aureus* is responsible for many hospital-associated and community-acquired infections. The emergence of methicillin-resistant *S. aureus* (MRSA) and vancomycin resistant *S. aureus* strains have prompted research into staphylococcal vaccines and preventive measures. Staphylococcal clumping factor A (ClfA), iron surface determinant B (IsdB) and gamma hemolysin B (HlgB) are key virulence factors and opsonic activity plays an important role in successful vaccine development against *S. aureus* infections.

**Objectives**

In this report, the BALB/c mice were immunized with a recombinant protein (CIH) composed of selected antigenic regions of ClfA, IsdB and HlgB of *S. aureus*. After the immunization, opsonophagocytic activity assay (OPK) was performed to all serum samples.

**Methods**

After exposing the opsonized bacteria with peritoneal macrophages, The phagocytic capacity of macrophages exposed with serum from mice immunized with recombinant protein CIH showed a significant increase as compared with macrophages exposed with serum from mice immunized with PBS ( $p=0.002$ ).

**Conclusions**

Our finding indicated, that immunization of BALB/c mice with recombinant protein CIH promotes the phagocytosis of *S. aureus* and enhances the clearance of the *in vivo* pathogen.



## **FEMS-2224**

### **New antimicrobials for resistant organisms**

#### **PERSEA AMERICANA GLYCOLIC EXTRACT: IN VITRO STUDY OF ANTIMICROBIAL ACTIVITY AGAINST CANDIDA ALBICANS BIOFILM AND CYTOTOXICITY EVALUATION**

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#### **Background**

Due to the resistance of microorganisms to antibiotics, studying alternative methods such as plant extracts becomes very important.

#### **Objectives**

Evaluating the *in vitro* antifungal activity of *Persea americana* extract on *C. albicans* biofilm and its cytotoxicity in macrophage culture (RAW 264.7).

#### **Methods**

For the determination of the minimum inhibitory concentration (MIC), microdilution in broth (M27-A2 standard) was performed. Thereafter, the concentrations of 12.5, 25, 50, 100 and 200 mg/mL (n=10) with 5 min exposure were analyzed on mature biofilm in microplate wells for 48 h. Saline was used as control (n=10). After treatment, biofilm cells were scraped off and dilutions were plated on Sabouraud dextrose agar. After incubation (37°C /48 h), the values of colony forming units per milliliter (CFU/mL) were converted to log10 and analyzed (ANOVA and Tukey Test, 5%). The cytotoxicity of the *P. americana* extract was evaluated on macrophages by MTT assay.

#### **Conclusions**

Results: The MIC of the extract was 6.25 mg/mL and with 12.5 mg/ml there was elimination of 100% of planktonic cultures. Regarding the biofilms, a significant reduction (p

Conclusion: *P. americana* extract showed antifungal activity against *C. albicans* biofilm, whereas the concentration of 50 mg/mL presented the best results.



**FEMS-0882**

**New antimicrobials for resistant organisms**

**EVALUATION OF MYRTUS COMMUNIS AND ZIZIPHUS LEAVES  
ANTIBACTERIAL ACTIVITY ON IMP-TYPE METALLO-BETA-LACTAMASE  
PRODUCING PSEUDOMONAS AERUGINOSA STRAINS**

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**Background**

*Pseudomonas aeruginosa* is one of the leading nosocomial pathogens. In recent years, inappropriate use of antibiotics is the cause of untreatable 'superbugs' and high death rates in clinics worldwide. One of the mechanisms of resistance to carbapenem antibiotics in *P. aeruginosa* is metallo- $\beta$ -lactamases (MBLs) production that hydrolyzes all carbapenems that are the last generation of drugs for treatment of infections caused by MDR *P. aeruginosa*.

**Objectives**

The aim of this study is evaluating the effects of *Myrtus communis* leaves and *ziziphus* leaves methanolic extract on IMP-Type metallo-beta-lactamase producing *P. aeruginosa* strains.

**Methods**

This experimental study was carried out on hospitalized burn patients during 2012-2013. Antibiotics and extracts susceptibility tests were performed by disc diffusion and broth microdilution methods. MBL detection was performed by Combination Disk Diffusion Test (CDDT). The bla(VIM) and bla(IMP) genes were detected by PCR and sequencing methods.

**Conclusions**

It was found that among 83 imipenem resistant *P. aeruginosa* strains, 48 (57.9%) were MBL producers. PCR and sequencing methods proved that these isolates were positive for blaIMP-1 genes, whereas none were positive for bla(VIM) genes. All MBL-producing *P. aeruginosa* were resistant to Meropenem, Imipenem, Ceftazidime, Amikacin, Tobramycin, Ciprofloxacin, Aztreonam, Piperacillin/Tazobactam, Ceftriaxone, Cefepime and Carbenicillin. The result of MIC for imipenem was 128( $\mu$ g/ml) for all strains. The MIC (mg/ml) and MBC (mg/ml) results of *Myrtus*

*communis* was 6.25 and 12.5 (mg/ml) for all isolates, *respectively* Although the ziziphus leaves methanolic extract represent any significant result on this isolates.

**FEMS-2578**

**New antimicrobials for resistant organisms**

**IDENTIFICATION AND CHARACTERIZATION OF A NOVEL CRYPTIC CATIONIC ANTIMICROBIAL PEPTIDE FROM THE THIRD DOMAIN OF LIFE**

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**Background**

The huge increasing of bacterial strains resistant to classical antibiotics is threatening the public health security, thus the need of developing new antimicrobials has become critical. Cationic Antimicrobial Peptides (CAMPs) represent suitable alternatives, since they do not lead to selection of resistant strains. Indeed, CAMPs act by disrupting the membrane integrity and/or by altering its potential, so that, to acquire resistance bacteria need to remodulate the membrane composition. Such modifications could be detrimental to bacterial cells, thus dramatically reducing the onset of resistant strains.

**Objectives**

Although proteins involved in the immune response of multicellular eukaryotes represent a natural source of CAMPs, new methodologies for their identification are required to encounter the rising demand of more active and stable CAMPs.

**Methods**

A powerful bioinformatics tool has been recently set up and used to predict the antibacterial activity of cryptic CAMPs (i.e. embedded in the primary structure of proteins). Given their hydrophobic/cationic nature, sequences of transcription factors were chosen as source of choice for the discovery of new cryptic CAMPs. In particular, our analysis focused on extremely stable proteins from thermophilic *Archaea*.

**Conclusions**

Among the identified CAMPs, one from the DNA-binding protein Stf76 (here named PepC) has been structurally and functionally characterized. This peptide has the capability of inducing both membrane fusion and pore formation. Interestingly, the DNA-binding activity was retained opening the way to speculate about novel mechanisms of action. Moreover, its antimicrobial activity has been proved on clinical strains isolated from patients affected by cystic fibrosis.

**FEMS-0983**

**New antimicrobials for resistant organisms**

**ANTAGONISTIC ACTIVITY OF MARINE SPONGE-ASSOCIATED BACTERIA  
COLLECTED AT WIMEREUX (FRANCE) AGAINST CLINICAL STRAINS**

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**Background**

Marine sponges are an important -and yet understudied- reservoir of new antimicrobials, a majority of which are produced by associated microbes.

**Objectives**

We tested the antibacterial activity of 110 bacterial strains isolated from two sponges (*Halichondria panicea* and *Hymeniacidon perelvis*) collected off rocky substrate in the low intertidal zone at Wimereux, France.

**Methods**

Strains were isolated on various media (R2A, Brain Heart Infusion (BHI)-agar and Marine agar) and tested against three target clinical strains : *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 2913. Antagonistic activity was measured in double layer agar assays.

**Conclusions**

Fifty strains (45%) were able to inhibit the growth of at least one clinical strain (most often *S. aureus*). Antibacterial-producing strains belonged to Gamma-*Proteobacteria* (88%, mostly *Pseudomonas* spp.) and *Firmicutes* (12%, among which *Lactococcus* spp. and *Vagococcus* spp.). The production of antimicrobial substances turned out to be modulated by the medium formulation, its salinity (tested from 0.5 to 3.5%) and the temperature used in antagonism assays (tested from 8 to 25°C). The best results were obtained with BHI-agar, the lowest salinity and highest temperature tested. In all cases, antimicrobial substances were not soluble, as the antagonistic activity was lost when cultures were filtered on 0.2 mm-pore membranes. This study confirmed the potential of sponge-associated microbes for the production of antibacterial substances.

## **FEMS-1787**

### **New antimicrobials for resistant organisms**

#### **ISOLATION AND CHARACTERIZATION OF NOVEL BACTERIOPHAGES WITH LYTIC ACTIVITY AGAINST MULTIDRUG RESISTANT PATHOGENS**

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#### **Background**

With the emerging threat of infections caused by multidrug-resistant bacteria and scarce prospects of newly introduced antibiotics in the future, bacteriophages (phages) are currently being reconsidered as alternative therapeutics. However, the full breath of phage diversity suitable for treatment of bacterial infections is still largely unexplored.

#### **Objectives**

This study aims at providing an overview of the general occurrence of bacteriophages (with therapeutic potential) in natural environments and at devising strategies for their rapid characterization and classification at molecular level.

#### **Methods**

Novel phages were isolated from various aquatic systems (e.g. from general and hospital wastewater, activated sludge samples from sewage plants, streams, rivers, ponds and lakes). Environmental samples were pre-incubated with clinical isolates of multidrug-resistant bacteria. Enriched bacteriophages were subsequently obtained with the double agar layer plaque assay. "PhiSigns"

(<http://www.phantome.org/phisigns/>) was used for phage identification based on the detection of signature genes. Genomic comparison of phages was performed based on digitized fluorescent restriction-fragment-length-polymorphism-analysis (fRFLP).

#### **Conclusions**

For roughly 50% of tested bacterial strains lytic bacteriophages were found against multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Enterobacter* sp. with waste water samples harbouring the highest phage diversity. By contrast no bacteriophages were isolated against multidrug-resistant *Staphylococcus aureus* and *Acinetobacter baumannii*. PhiSigns combined with fRFLP proofed to be a useful tool for rapid phage identification, which is the base for their proper selection for ultimate whole genome sequencing. *S. aureus* and *A. baumannii* phages require additional isolation steps such as FeCl<sub>3</sub>-precipitation and/or use of



subinhibitory antibiotic-concentrations for enhancing plaque visibility on agar plates.

**FEMS-2514**

**New antimicrobials for resistant organisms**

**ANTIFUNGAL ACTIVITY OF ESSENTIAL OIL FROM ABIES HOLOPHYLLA  
MAXIM AGAINST DERMATOPHYTES**

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**Background**

Dermatophytes are well known for one of world-wide cause of diseases such as tinea pedis, tinea cruris, and tinea corporis. Dermatophytes infections are increasing of taking antifungal agents, accordingly increased side effects like toxicity and tolerance. That was why searches on natural antifungal medicine have motivated. Many kinds of woody essential oils have antifungal activity, especially essential oils of conifers. *Abies holophylla* Maxim (AH) is widespread in East Asia and its essential oil was known to have effective antifungal actions against *Aspergillus fumigatus*.

**Objectives**

Therefore, this study was to investigate the antifungal activity of AH essential oil against dermatophytes, such as *Epidermophyton floccosum*, *Trichophyton mentagrophytes* and *Trichophyton rubrum*, and to determine the potential effective compound as dermatitis treatment.

**Methods**

To evaluate the potential antifungal activities of AH essential oil and its fractions, Minimum Inhibitory Concentration (MIC) measurement and agar dilution method were used with morphological observation. Also, their major constituents were analyzed by GC/MS. The morphological changes of the dermatophytes exposed to the AH essential oil were observed by electron microscopes.

**Conclusions**

As the results, the highest activities were identified in the fraction containing borneol, limonene, 3-carene, and camphene. Also, morphological observation using electron microscopes (SEM and FEM) showed a dramatic changes of cell wall of dermatophytes exposed to the AH essential oil. In conclusion, AH essential oil and its constituents were expected to be used as antifungal agent or raw material for dermatitis therapy.

**FEMS-1646**

**New antimicrobials for resistant organisms**

**IN VITRO ANTI-STAPHYLOCOCCAL ACTIVITY OF SECONDARY METABOLITES FROM DROSERA BINATA COMBINED WITH SILVER NANOPARTICLES**

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**Background**

Antibiotic resistant *Staphylococcus aureus* is a serious nosocomial pathogen and the major cause of life threatening infections, like burn wound infections. There is an urgent need to overcome antibiotic resistance by developing new antimicrobial treatment. The use of active plant secondary metabolites simultaneously with silver nanoparticles (AgNPs) against *S. aureus* is based on multi-target strategy.

**Objectives**

The aim was to find an alternative anti-staphylococcal treatment based on the combination of *Drosera binata* secondary metabolites and silver nanoparticles.

**Methods**

Plant extracts were obtained from *in vitro* cultured *D. binata* tissues. Antibacterial activity was determined by Broth Microdilutions Method. Checkerboard Titration technique was used to study interactions between AgNPs and extracts or secondary metabolites. Composition of the extract was established by the HPLC-MS analysis. The MTT Assay was used to evaluate cytotoxicity of antimicrobial agents towards *in vitro* cultured human keratinocytes.

**Conclusions**

Both silver nanoparticles and the *D. binata* extract possess antibacterial activity towards various *S. aureus* strains regardless of their antibiotic resistance. The synergistic bactericidal activity was observed when AgNPs and plant extracts were combined. This allowed to significantly reduce the bactericidal concentrations of AgNPs and the extract by 50% and 97%, respectively. Among all extract constituents only the chlorine derivative of plumbagin (ChPL) showed synergistic bactericidal activity with AgNPs. Moreover, reduced bactericidal concentrations of mixtures of

AgNPs with the extract or ChPL were observed to be non-cytotoxic towards human keratinocytes. Obtained results indicate that combination of AgNPs and secondary metabolites from *D. binata* tissues may provide an alternative antimicrobial treatment.

**FEMS-1866**

**New antimicrobials for resistant organisms**

**ANTIBACTERIAL AND ANTIFUNGAL ACTIVITY OF NEWLY SYNTHESISED  
BENZOXABORoles, BENZOSILOXABORoles AND PYRIDOXABORoles**

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**Background**

Benzoxaboroles, and related compounds, are a particularly interesting group of synthetic agents. Their diverse biological activities and widespread applications in medicinal chemistry have undergone intense scientific investigation.

**Objectives**

We have examined whether new benzoxaborole-based derivatives and their analogues possess antimicrobial activity and are substrates for multi-drug-resistance efflux pumps.

**Methods**

The antibacterial and antifungal activities of newly synthesised compounds: 3 benzoxaborole-, 3 benzosiloxaborole- and 6 pyridoxaborole-based derivatives were evaluated. Antimicrobial activity was screened using the disc-diffusion test (0.4mg of the derivative was applied to a filter paper disc). The MIC and MBC values were estimated according to CLSI and EUCAST recommendations. During determination of MIC values  $\pm$  PAbN (80mg/L), the concentrations of tested compounds in the broth medium ranged: 0.39-400mg/L. The 19 reference ATCC strains of Gram-positive, Gram-negative bacteria and yeast were used in this study.

**Conclusion:**

Generally, the most potent antibacterial and antifungal activities were found for fluorine substituted benzosiloxaboroles – analogues of benzoxaboroles possessing a silicon atom for carbon substitution at the 3-position (MICs 25-400mg/L and 0.78-100mg/L, respectively). Interestingly, the B(OH)<sub>2</sub>-substituted 3-phenylbenzoxaboroles were found to be the most potent against Gram-positive cocci: *Staphylococcus* sp. (MICs 12.5-50mg/L), *Enterococcus* sp. (MIC 200mg/L) and yeasts (MIC 50mg/L). Furthermore, we demonstrated that benzoxaboroles were removed by efflux pumps present in Gram-negative bacteria. Of note, only two pyridoxaborole-based compounds were found to be weakly active against *Staphylococcus* sp.

These data demonstrate structure-activity relationships of the tested derivatives and

highlight the need need for futher investigations into the antimicrobial properties of new benzoxaboroles and related compounds.

## FEMS-2473

### New antimicrobials for resistant organisms

#### APPLICATION OF A HIGH-THROUGHPUT ASPERGILLUS FUMIGATUS CELL WALL STRESS REPORTER SYSTEM: IDENTIFICATION OF SYNTHETIC PEPTIDES INCREASING THE SENSITIVITY FOR ANTIFUNGAL MEDICINES

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#### Background

Increased resistance to currently used antifungal compounds and the fact that these agents are often harmful to man and environment have resulted in a growing demand for new antifungals, which selectively act on cellular processes that are unique to fungi. To meet this demand, we have established in *A. niger* a luciferin/luciferase based reporter system for high-throughput screening of natural products for identification of potential new antifungal drugs.

#### Objectives

Our system allows us to identify compounds that specifically inhibited the fungal cell wall biosynthesis.

#### Methods

Recently the system has been developed for *A. fumigatus* and non-*Aspergillus* species. The system was validated by analyzing its performance with several antifungal drugs which are commonly used in the clinic like Caspofungin, Amphotericin B, Voriconazol (V fend) as well as Nikkomycin. A dose response behavior of the compounds could be demonstrated together with in time different types of stress responses. Importantly, whether the compound tested is fungistatic or fungicidal could be clearly indicated.

Subsequent analysis of different synthetic antimicrobial peptides, HTX1-4, revealed also a moderate effect on cell wall stress induction indicated by an increase in Lux activity.

#### Conclusions

Interestingly, incubation of these peptides at sublethal concentrations together with some of the antifungal medicines showed a considerable increase in lux activity

and a significant increase in sensitivity of *A.fumigatus* for these medicines. Growth test with sublethal concentrations of HTX-2 on EBV-LCL, K562 cells and PHA-blasten, and also cytotoxicity- and genotoxicity tests with NCTC2544 cells did not show any negative/toxic effects on these human cell lines.



## **FEMS-3022**

### **New antimicrobials for resistant organisms**

#### **PRODUCTION OF NEW-TO-NATURE HYPERMODIFIED LANTIBIOTICS**

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#### **Background**

Lantibiotics are potent peptide antimicrobials active against Gram-positive bacteria, including multidrug resistant pathogens. They display various posttranslational modifications, with lanthionine rings and dehydroamino acids as key signatures of this family of antimicrobials. In addition, some lantibiotics contain other enzymatic modifications that have a role in their activity.

#### **Objectives**

Our purpose is to combine different enzymes from diverse lantibiotic production systems in order to hypermodify the model lantibiotic nisin as a proof-of-principle. This can set a methodology for the design and modification of other (antimicrobial) peptides.

#### **Methods**

We have combined, in either *Lactococcus lactis* or *Escherichia coli*, the modification machinery of nisin (the dehydratase NisB, the cyclase NisC) with the hydroxylases MibO and CinX from the microbisporicin and the cinnamycin machineries, respectively; the C-terminal decarboxylase GdmD from the gallidermin cluster; and the reductase LtnJ from the lactacin 3147 system.

#### **Conclusions**

We have generated nisin derivatives containing hydroxyproline, hydroxyaspartate, a C-terminal aminovinyl-cysteine, or D-alanine in their structure. Moreover we have confirmed that these additional enzymes do not require leader peptide recognition to modify their substrate.

Our results provide an insight for the application of Synthetic Biology for the design and *à la carte* modification of (antimicrobial) peptides.

**FEMS-2648**

**New antimicrobials for resistant organisms**

**THERAPEUTIC MONOCLONAL ANTIBODIES AGAINST THE GLOBALLY SPREAD MULTI-DRUG RESISTANT ESCHERICHIA COLI CLONE ST131-O25B:H4**

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**Background**

The globally spread multi drug resistant *E. coli* clone ST131-O25b:H4 is responsible for a significant proportion of multi-drug resistant extraintestinal infections. By definition, isolates of this clone express the O25b antigen, whose unique structure has been resolved recently.

**Objectives**

We aimed to develop humanized monoclonal mAbs against the O25b antigen that may provide an alternative adjunct or stand-alone therapeutic option against this *E. coli* lineage.

**Methods**

Murine mAbs against the unique O25b antigen were generated by standard hybridoma technique. Following a primary screening, selected antibodies were subjected to humanization. Specificity and binding characteristics were assessed by immunoblots and ForteBio using purified antigens. Surface staining of ST131-O25b isolates irrespective of the capsular type expressed was shown by flow cytometry. Upon surface binding, the humanized antibodies induced complement mediated bactericidal activity *in vitro*. Passive immunization with the humanized mAbs elicited high level of protection at low mAb doses in a murine model of bacteremia. All whole cell assays were performed with bacteria grown in standard culture medium as well as in depleted pooled human serum in order to mimic *in vivo*-like conditions.

**Conclusions**

Humanized mAbs against *E. coli* O25b antigen were developed that elicited bactericidal activity *in vitro* and showed protection *in vivo*. Prophylactic passive immunization of colonized individuals or adjunct therapy of infected patients by mAbs may replace/substitute antibiotic therapy against this drug resistant clone. Relevant cases could be identified by the co-developed companion diagnostic tool.

**FEMS-0880**

**New antimicrobials for resistant organisms**

**FREE AND IMMOBILIZED PHOTSENSITIZERS FOR ERADICATION OF PATHOGENIC BACTERIA**

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**Background.** The problem of controlling microbial infections remains one of the most serious challenges faced by mankind, despite the development of very effective antimicrobial agents. Bacterial resistance to antibiotics, which for decades were considered to be capable of combating any bacterial infection, actually destroyed hopes for combating pathogenic bacteria. One of alternatives to antibiotic treatment is photodynamic antimicrobial chemotherapy based on visible light-activation of dyes called photosensitizers. In the dark, these photosensitizers are non-toxic or have low toxicity. No bacterial resistance to photosensitizers has been reported to date.

**Objectives.** The present report is dedicated to the antibacterial activity of the photosensitizers Rose Bengal and Methylene Blue against Gram-positive *Staphylococcus aureus*. The antimicrobial activity of both free and immobilized photosensitizers was examined.

**Methods.** Rose Bengal and Methylene Blue were immobilized on polystyrene, polycarbonate and poly(methyl methacrylate) by dissolution in chloroform with further evaporation of the organic solvent and the formation of thin polymeric films. Immobilization of these photosensitizers on polyethylene and polypropylene was performed by dissolution in the melted polymers. The free and immobilized photosensitizers showed high efficiency in eradication of *S. aureus* as tested by the MIC, the colony-forming units count and measurement of the inhibition zone.

**Conclusions.** Photodynamic inactivation of *S. aureus* by Rose Bengal and Methylene Blue opens prospects for developing an alternative to antibiotic treatment of bacterial infections.

**FEMS-2719**

**New antimicrobials for resistant organisms**

## **ANTIMICROBIAL PEPTIDES OF MARINE ORIGIN AND THEIR MOLECULAR CHARACTERIZATION**

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### **Background**

Marine organisms are continuously exposed to microbial population and are equipped with effective immune molecules to combat microbial invasion. Antimicrobial peptides are important components of innate defense mechanisms exhibited by animals. These organisms produce a range of AMPs varying greatly in size, structure and other physicochemical properties. Investigating novel antimicrobial peptides from marine organisms can provide new insight into the immune response of these organisms and a possibility of discovering new and effective drugs in medicine/aquaculture.

### **Objectives**

Screening marine organisms for discovering novel antimicrobial peptides

### **Methods**

Total RNA was extracted from haemocytes and cDNA was synthesized. PCR amplification was done using gene specific primers for AMPs. Amplicons were cloned and sequencing of the inserts were done at SciGenom, India. Homology search was performed by using BLASTn and BLASTp at NCBI and physio- chemical characterization by ProtParam Tool. Phylogenetic tree was constructed based on amino acid sequences by Neighbor joining method using MEGA 5.0

### **Conclusions**

Four novel ALF isoforms were characterized, among which two were identified from crabs (*Scylla serrata* and *Portunus pelagicus*) and two from shrimp (*Fenneropenaeus indicus*). Four novel isoforms of crustins were also identified and characterized from *Scylla serrata*, *Scylla tranquebarica*, *Portunus pelagicus* and *Fenneropenaeus indicus*. Two penaeidin isoforms were characterized from *Fenneropenaeus indicus* and *Penaeus monodon* and a hepcidin from *Chlorophthalmus bicornis*. The marine environment, with its enormous biodiversity remains a largely untapped reservoir of bioactive peptides. Recombinant expression and bioactivity testing of these peptides

will definitely contribute to the design and formulation of potential peptide drugs for application in medical/ aquaculture industry.

## **FEMS-3083**

### **New antimicrobials for resistant organisms**

#### **RESEARCHANT HUB: THE FIRST ONLINE PLATFORM FOR EXPERIMENTAL COMPOUND SHARING**

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#### **Background**

Today we witness the global health threat of antimicrobial resistance. This phenomenon relies largely on the erosion of the effective antimicrobials and has drawn the attention of researchers from the traditionally used antimicrobials to the so-called experimental compounds.

Experimental compounds are bioresearch compounds that have been subject to a certain degree of development, but have been put away either due to project deprioritization or lack of effectiveness against the application in question. They may, however, be very valuable in other applications; for instance, in diseases sharing similar biochemical pathways. These compounds are a rich source of new and, possibly, better antimicrobials. The question is: How can researchers gain access to them? We will discuss our efforts in developing a compound sharing facility and how we will collaborate with the community to facilitate open-innovation models for the antimicrobial compounds discovery.

#### **Objectives**

Our goal is to advance research in the field of antimicrobial resistance by re-purposing rare and one-of-the-kind experimental compounds to the whole of academic community. For that, we developed ResearchAnt Hub (RAH). RAH is powered by the ResearchAnt Foundation, which is a not-for-profit organization. RAH is a repository and a platform that fosters researchers worldwide to use and share their experimental compounds.

#### **Methods**

Our repository will contain thousands of experimental compounds that were developed or manufactured by privately- and academically-led researchers during the course of their work.

#### **Conclusions**

RAH is a unique not-for-profit open-innovation initiative. It represents a major step towards the discovery of new drugs to counteract today's ever-increasing antimicrobial resistance problem.



## **FEMS-1395**

### **New antimicrobials for resistant organisms**

#### **PSEUDOMONAS AERUGINOSA STRAINS PRODUCING ANTIMICROBIAL COMPOUNDS ISOLATED FROM HOSPITAL ENVIRONMENTS**

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#### **Background**

Antibiotic resistant human pathogens are diminishing our ability to treat infections and novel antimicrobials are urgently needed to replace our current arsenal of antimicrobials. *Pseudomonas aeruginosa* strains have been shown to produce antimicrobial compounds including bacteriocins, pigments and secondary metabolites that show promise as novel antimicrobial therapeutic agents.

#### **Objectives**

To assess the antimicrobial activity of four environmental *P. aeruginosa* isolates from University College London, Hospital (UCLH).

#### **Methods**

Four *P. aeruginosa* isolates were obtained from various sites in UCLH. Cross streak assays using indicator organisms including *Escherichia coli*, *Staphylococcus aureus* and *Salmonella typhimurium* were conducted to determine the spectrum of inhibition of these *P. aeruginosa* strains. A spot-on lawn assay was used to compare the ability of each strain to inhibit the growth of *S. aureus*. To determine if the *P. aeruginosa* strains secreted compounds that were inhibiting *S. aureus*, this indicator strain was grown in cell free media that the *P. aeruginosa* strains were previously grown in.

#### **Conclusions**

Competitive hospital environments may be a rich source of bacteria producing novel antimicrobials. The results of the above experiments show that these four hospital environment acquired *P. aeruginosa* isolates are capable of inhibiting a wide range of bacteria. Some strains had a greater ability to inhibit *S. aureus* than others. All of the *P. aeruginosa* strains secreted inhibiting compounds into the media they were grown in. We are currently characterising the genetic and biochemical basis of these compounds.



**FEMS-0939**

**New antimicrobials for resistant organisms**

**EFFECT OF BACTERIOCIN PRODUCED BY STAPHYLOCOCCUS EPIDERMIDIS AGAINST MRSA**

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- Background: Staphylococcus aureus including methicillin-resistant S. aureus (MRSA) is recognized as a globally important pathogen in food-borne disease. Therefore bacteriocins have been focused on new weapon that are considered safe and effective substances as food additives to prevent food-borne pathogens.
- Objectives: To control MRSA of agricultural product, identify and characterize a antimicrobial peptide (bacteriocin) produced by Staphylococcus epidermidis.
- Methods: S. epidermidis were isolated from leafy green vegetables and identified by API Staph ID test and 16s rRNA sequence analysis. Antimicrobial activities of S. epidermidis against antibiotic resistant S. aureus and other Gram (+) bacteria were tested using agar diffusion assay. Purification of the active compound was achieved using a combination of ammonium sulfate precipitation, dialysis and reverse-phase high-performance liquid chromatography (RP-HPLC). The effects of heat, pH and enzymes on bacteriocin activity were determined using S. aureus CCARM3723 as indicator organism.
- Conclusions: S. epidermidis S-199 strain showed broad and strong antimicrobial activity. Purified antimicrobial peptide was heat-labile but stable in a wide range of pH. And the antimicrobial peptide showed resistance to  $\alpha$ -amylase, lipase, and RNase, and DNase. However, antimicrobial activity was lost after trypsin, pronase E, pepsin,  $\alpha$ -chymotrypsin, proteinase K, and papain treatment.

## **FEMS-1587**

### **New antimicrobials for resistant organisms**

#### **ROLE OF PRODIGIOSIN AS A MICROBICIDE**

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#### **Background**

Number of microbial species are metabolically wired to produce some pigments constitutively. Prodigiosin is such a pigment having prodigious biological activities in accord to its name. Microbicides are compounds that can be applied inside the vagina or rectum to protect against sexually transmitted infections (STIs). Discovery of drugs, targeting multiple steps in multiple diseases is of great interest.

#### **Objectives**

To evaluate prodigiosin for its microbicide potential and study its mechanism of actions in restricting sexually transmitted infections like *E. coli*, *C. albicans*, HSV, HIV, *Trichomonas vaginalis*. ii) To check cytotoxicity of prodigiosin against human cells.

#### **Methods**

96 well plate assay used for antibacterial assay followed by its biochemical investigations after treatment with prodigiosin at LC<sub>30</sub> concentration, cell associated and cell free assays were used to check anti HIV-1 (CXCR4 and CCR5) potential, while ONPG for HSV, cytotoxicity of prodigiosin was determined by MTT assay.

#### **Conclusions**

Prodigiosin induces leakage in bacterial cell membrane and simultaneously inhibits activity of few essential enzymes. Prodigiosin inhibits viral entry and cell fusion for HSV and HIV (IC<sub>50</sub>- 0.01 µg/ml). It also inhibited *T. vaginalis* (1.2 µg/ml). The use of pigment found on safer side against human cells (PBMC (78±3.1 µg/ml) and HeLa cells (70.2±5.3 µg/ml)). One compound having all these activities is a remarkable feature. Prodigiosin can be formulated as a microbicide (drug) and applied on the affected area with a cream base so as to treat the STIs. In conclusion prodigiosin can be used in treatment modality for protection from STIs.



**FEMS-0701**

**New antimicrobials for resistant organisms**

**REGULATORY NETWORKS AND ECOLOGICAL CUES THAT CONTROL  
ANTIBIOTIC PRODUCTION BY ACTINOMYCETES**

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**Background**

Actinomycetes are prolific producers of natural products, including anticancer compounds, antifungals and antibiotics. The treasures that lie hidden in the actinomycete genomes may well be our final resource in the battle against the rapidly emerging infectious diseases associated with multi-drug resistance.

**Objectives**

We aim to understand the triggers and cues that elicit antibiotic production in the soil as well as in the laboratory. This knowledge is then translated into technologies for the activation and mining of silent pathways.

**Methods**

To uncover novel antimicrobials, we use a combination of ecological insights, systems biology, NMR-based metabolomics and genome mining to uncover the regulatory mechanisms that control antibiotic production. We then apply this for the discovery of novel antimicrobials.

**Conclusions**

One major control system revolves around the nutrient sensory protein DasR, which pleiotropically controls antibiotic production. Many other control systems are undoubtedly in place to allow actinomycetes to respond appropriately to challenges by competitors and fluctuations in nutrient composition in the habitat. Furthermore, once a bioactivity is elicited under specific growth conditions, novel approaches are needed to rapidly identify the bioactivity of interest and link it to a specific gene cluster. Molecular and ecological insights to elicit antibiotic production will be discussed, and examples of novel antibiotics identified by these approaches are presented.

**FEMS-1522**

**New antimicrobials for resistant organisms**

## **ACTIVITY ANALYSIS OF LYSOSOMES AS A FUNCTIONAL CELL ORGANELLE IN *SACCHAROMYCES CEREVISIAE***

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### **Background**

Lysosomes are functional cell organelles which have antimicrobial and anticancerous activity. Lysosomal enzymes are easily integrated or released when exposed to some stimuli factors in all eukaryotic cells.

### **Objectives**

In order to verify features special function of lysosomes, several conditions were used to stimulate *in vitro* functions of lysosomes. In addition, we confirm lysosomal safety and activity *in vivo* for using antimicrobial and anticancerous materials.

### **Methods**

Proteomic analysis of lysosomal enzymes in response to the enhanced and repressed condition for *in vitro* activity using 2-DE. Among up-regulated protein dots response to oxidative stress, the dot with highest increase was chosen to overexpress in *Saccharomyces cerevisiae* and then confirm the lysosomal activity. In addition, we injected lysosomes into rat model infected with bacteremia, and then analysis bacterial growth in blood of rat according to the reaction time.

### **Conclusions**

The antimicrobial and anticancerous activity of lysosomes was enhanced in cloned *S. cerevisiae in vitro* assay. In addition, it is safe to inject lysosome into normal rat. Moreover, when being injected into rat model infected with bacteremia, lysosome showed its ability to inhibit bacterial growth in blood. Therefore, increases in endogenous levels of lysosomes may have various applications such as antimicrobial agents and apoptosis-inducing materials for cancer cells and it is very promising to use the organelles to improve *in vitro* and *in vivo* functions.

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**FEMS-1529**

**New antimicrobials for resistant organisms**

**EFFECT OF ACID TREHALASE (ATH) ON IMPAIRED YEAST VACUOLAR ACTIVITY**

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**Background**

Acid trehalase (ATH) has been suspected to have two different localizations, vacuolar lumen and cell surface, among which the localization at cell surface is believed to be responsible for extracellular trehalose utilization.

**Objectives**

A lot of efforts were made to confirm the exact localization of ATH in yeast cells. Along with this, the trafficking pathway responsible for the delivery of ATH into yeast vacuoles was also studied with the purpose to provide better control on the transportation of this protein in yeast cells.

**Methods**

ATH was overexpressed in yeast cells grown on trehalose-containing medium to observe the impact of this enzymes on yeast vacuolar activity. After that, 4 groups of typical proteins representing the 4 main trafficking pathways in yeast were also selected to analyze their expression pattern via 2DE.

**Conclusions**

The overexpression of ATH was observed to decrease vacuolar activity. Moreover, ATH was confirmed again to locate in both cell surface and vacuoles. Finally, it is interesting to discover that on medium containing trehalose, the ATH's localization at cell surface, but not vacuoles, is prioritized to utilize extracellular trehalose for cell growth. The mechanism behind that is suggested via the down regulation of MVB pathway that sorts ATH into vacuoles.

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**FEMS-1644**

**New antimicrobials for resistant organisms**

## **ANTIMICROBIAL AND ANTI-BIOFILM ACTIVITY OF SELENIUM AND TELLURIUM NANOPARTICLES OF BACTERIAL ORIGIN**

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### **Background**

In the last few decades, the emergence of bacterial resistance to antibiotics has become a common phenomenon. As a consequence, the effectiveness of antibiotic treatment of bacterial infection has progressively decreased. Therefore, it's necessary to develop and test new antimicrobial compounds against multidrug-resistant bacteria. Recently, the employment of metallic nanoparticles has emerged as an alternative to the use of organic compounds as antimicrobial agents: different metals and metalloids nanoparticles have indeed shown a promising bactericidal capability.

### **Objectives**

The aim of the present study was to evaluate the antimicrobial activity of selenium and tellurium nanoparticles of bacterial origin against both planktonic and biofilm culture of three pathogenic strains: *Escherichia coli* JM109, *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus* ATCC 25923.

### **Methods**

To measure the antimicrobial activity of these nanoparticles we determined both the minimum biocidal concentration (MBC) and the minimum biofilm eradication concentration (MBEC) using the MBEC assay™. We also observed the effect of increasing concentrations of nanoparticles on biofilm structure using Confocal Microscopy (CLSM).

### **Conclusions**

Our results indicate that both Se<sup>0</sup> and Te<sup>0</sup> nanoparticles possess antimicrobial and biofilm eradication activity. In particular Se<sup>0</sup> nanoparticles exhibited antimicrobial activity at lower concentration. The activity seemed to be dependent on the dimension of the nanoparticles: indeed, the highest activity was shown by the nanoparticles smaller in size. The key observation is that bacteria grown in biofilm mode didn't exhibit a higher level of resistance against the nanoparticles antimicrobial action. Results described in this study suggest a possible application of these nanoparticles as an effective antimicrobial agent.





## **FEMS-1881**

### **New approaches for typing**

#### **AN INTEGRATED PLATFORM FOR HIGH-THROUGHPUT WHOLE GENOME MLST AND WHOLE GENOME SNP ANALYSIS.**

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### **Background**

With the increasing availability of next generation sequencing technology, whole genome sequencing (WGS) methods are increasingly being used for bacterial typing. Key challenge is the ability to rapidly extract the relevant information from large sequence data files.

### **Objectives**

We present two pipelines for high resolution WGS-based molecular typing: whole genome multilocus sequence typing (wgMLST) and whole genome single-nucleotide polymorphism analysis (wgSNP). Both strategies are compared using data from a *Staphylococcus aureus* outbreak.

### **Methods**

Using an in-house developed wgMLST schema that extends the core genome schema from Leopold *et al.* (2014), we apply two independent allele calling approaches, an assembly-free and a BLAST-based allele calling algorithm, to determine locus presence and detect allelic variants in a quality-controlled manner. The wgSNP pipeline, tuned to reduce false positives while maximizing resolution, detects SNP variants by mapping the WGS reads to a reference sequence internal or external to the data set.

For both methods, all calculation-intensive data processing steps are performed on the BioNumerics® Calculation Engine, deployed locally or in the cloud.

### **Conclusions**

The BioNumerics® 7.6 software and its integrated Calculation Engine offer a powerful platform where both wgMLST and wgSNP can be performed to provide a robust,

portable and high resolution picture of molecular typing data. The polyphasic approach allows for validation both between WGS analysis techniques and traditional techniques such as MLST or PFGE. We illustrate this by comparing core genome MLST (cgMLST), wgMLST and wgSNP, thus establishing a clear picture of the differences in resolution between those analysis techniques.

**FEMS-0553**

**New approaches for typing**

## **MOLECULAR INVERSION PROBES AND PYROSEQUENCING FOR SENSITIVE DETECTION OF MYCOBACTERIUM TUBERCULOSIS**

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### **Background**

Tuberculosis is a treatable disease, yet remains a major worldwide health problem. In the last 10 years approximately 80 million people contracted the disease. The development of techniques for rapid diagnosis based in molecular technologies would greatly facilitate worldwide efforts to prevent further spread of the disease. One potential diagnostic sequence, the DR locus, is present in all isolates of *M. tuberculosis* complex bacteria. It is useful for molecular typing of *M. tuberculosis* because of its fortuitous absence in non-tuberculosis strains of mycobacteria.

### **Objectives**

In this study, we attempted to combine the specificity of molecular inversion probe (MIP) technology with the sensitivity of pyrosequencing in order to detect a short conserved 18 bp sequence included in DR locus in 25 isolates of *M. tuberculosis*.

### **Methods**

DNA from 25 *M. tuberculosis* isolates were extracted and submitted to the MIP reactions. A MIP was designed against the DR sequence of *M. tuberculosis* strain H37Rv and the MIP reactions were performed; the MIPs were then submitted to pyrosequencing reactions.

### **Conclusions**

We design a molecular inversion probe for a specific region of *M. tuberculosis* genome. Probing this MIP with 25 *M. tuberculosis* genomic DNA and determining their sequences by pyrosequencing, we detect a common sequence among all strains of *M. tuberculosis*. Also, we determine the minimal amount of DNA needed (50 ng) in order to obtain a good readout. By introducing a modification on pyrosequencing

methodology we were successful in detecting M.tuberculosis DNA even in presence of a very small amount of DNA (500fg).

**FEMS-2810**

**New approaches for typing**

**TYPING OF ENTEROBACTERIACEAE BY ERIC-PCR, REP-PCR AND MALDI-TOF MS METHODS**

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**Background:**The fast and reliable fingerprinting methods for typing of *Enterobacteriaceae* are widely required in food microbiology for tracking sources and ways of food contamination. ERIC-PCR and REP-PCR genotyping use as markers specific DNA sequences (enterobacterial repetitive intergenic consensus sequences or repetitive extragenic palindromic sequences, respectively) and the changes of their dispersal, detectable by methods. In comparison MALDI-TOF MS phenotyping uses the conformity of protein profiles. As used typing markers mirror the differences among strains only partially, also compared to other features as the antibiotics resistance and biofilm formation, their combination could propose more precise approach.

**Objectives:**The aim was to compare the discriminatory power of ERIC-PCR, REP-PCR and MALDI-TOF MS fingerprinting methods for different *Enterobacteriaceae* isolates, also with respect to their antibiotics resistance and biofilm formation.

**Methods:**Fifty six isolates of *Enterobacteriaceae*, including genera *Escherichia*, *Enterobacter*, *Klebsiella*, *Serratia* and *Raoultella*, isolated from food and clinical sources in Czech Republic in 2007-2014, were genotyped by the same ERIC-PCR and REP-PCR methods (maximal length of analysed fragments 3000 bp). MALDI-TOF MS typing was done by the ethanol-formic acid extraction in Bruker Autoflex Speed MALDI-TOF mass spectrometer. The resistance for 12 antibiotics and the ability to form biofilm in different media at 25 °C were tested.

**Conclusions:** REP-PCR is more discriminative than ERIC-PCR in most species. The discriminatory power of MALDI-TOF MS phenotyping depends on the range of used protein peaks (e.g. application of specific intensity criteria) and the used clustering methods, but never gives the false positive results in comparison to the genotyping methods.

**FEMS-0688**

**New approaches for typing**

**USE OF MATRIX-ASSISTED LASER DESORPTION/IONIZATION- TIME OF FLIGHT (MALDI-TOF) MASS SPECTROMETRY FOR ROUTINE ANALYSIS IN A DRINKING WATER TREATMENT PLANT**

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**Background**

Drinking water treatment plants employ different treatments depending on source water quality to assure adequate chemical and microbiological quality according to the Directive 98/83/EC. The study of bacterial communities throughout the treatment plant could provide a basic understanding of the effects of water processing that can be used to improve the management in drinking water treatment plants.

**Objectives**

To assess the use of MALDI-TOF MS for routine analysis in a drinking water treatment plant to study bacterial diversity throughout the different treatments in comparison with other methods such as the PhenePlate® system, the API 20E strip and 16S rRNA gene sequencing.

**Methods**

A total of 366 colonies isolated from different points and seasons throughout the water treatment process including feed water (40), sand filtration (36), ultrafiltration (44) and reverse osmosis (191) were used in the study. The colonies were analyzed by MALDI-TOF MS by direct colony inoculation on the plate. The colonies were also biochemically fingerprinted using the PhenePlate® system, clustered according to their similarity and a representative strain was selected for 16S rRNA gene sequencing and API based identification.

**Conclusions**

The diversity decreased along the different treatments, with a total of 20 genera being detected. The use of MALDI-TOF MS was reliable compared to the PhenePlate® system and has the advantage of being faster and relatively cheap compared to other technologies such as the 16S rRNA sequencing. The API web was not sufficiently reliable for water monitoring since the identification of the majority of the strains failed compared to the other methods used.

**FEMS-1729**

**New approaches for typing**

**MOLECULAR DIVERSITY OF CANDIDA ALBICANS ISOLATED FROM IMMUNOCOMPROMISED PATIENTS, BASED ON MLST METHOD**

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**Background**

Multilocus sequence typing (MLST) is used as a highly discriminatory method for tracing sources and routes of transmission, and the genetic relatedness of isolates.

**Objectives**

The aim of the study was to assess the genetic diversity among *C. albicans* strains isolated from oropharynx and bronchoalveolar lavage (BAL) of immunocompromised patients.

**Methods**

A total of fourteen epidemiologically unrelated clinical isolates of *C. albicans* from three hospitals in northern Iran were tested. Seven loci of housekeeping genes were sequenced for all fourteen isolates.

**Conclusions**

The fourteen isolates were placed in 10 clonal clusters (CC) while two isolates were singletons, by eBURST analysis. Most of the isolates were belong to CC461 of eBURST analysis from the clade 11 and two isolates assigned to CC172 from the clade 15.

In conclusion, Genetic diversity is variable among unrelated strains obtained from different patients at different times and places, and epidemiologically unrelated strains usually are not genetically closely related and classify in different clusters

**FEMS-3008**

**New approaches for typing**

**MULTILOCUS SEQUENCE TYPING STRAINS OF YERSINIA  
PSEUDOTUBERCULOSIS ISOLATED IN RUSSIA**

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Background: The epidemic spread of a special clinical and epidemiological form of pseudotuberculosis, Far East Scarlet-Like Fever (FESLF) was firstly described in Russia (Far East, Vladivostok) in the late 1950s [1].

Objective: To characterize *Yersinia pseudotuberculosis* strains isolated in Russia (Far East, Siberia, Europe) in 1973-2014 years with the multilocus sequence typing (MLST) method to ascertain a position of the strains in a phylogenetic structure of the species.

Methods. Totally, 80 *Y. pseudotuberculosis* strains isolated from the stool of patients with clinical signs of FESLF (n=54), vegetables (n=11) and rodents (n=15) were included in the study. The MLST method developed by Achtman et al. (<http://www2.warwick.ac.uk/mlst>) was used. The MLST scheme is based on 7 house-keeping genes.

Conclusion. The majority of *Y. pseudotuberculosis* strains isolated from patients belonged to sequence type ST2 (85.2%, serotype 01b). Five and three clinical strains belonged to ST26 (9.3 %, serotype 01b) and ST32 (5.5%, serotype 03), respectively. *Y. pseudotuberculosis* serotype 01b strains isolated from vegetables belonged to ST2 (81.8%) and ST14 (18.2%). Strains isolated from rodents (01b serotype) belonged to ST2 (60%), ST42 (20%), ST64 (13,3%), ST32 (6,7%). Serotype O3 rodent isolates belonged to ST14 (5.3%). MLST results showed the *Y. pseudotuberculosis* ST2 serotype 01b clone prevails in Russia among all studied sources including patients, vegetables, and rodents.

1. Grunin I.I., Somov G.P., Zalmover I. Yu. Far East Scarlet- Like Fever // Military Medical Journal. 1960. № 8. P. 62–66.



**FEMS-2988**

**New diagnostic approaches**

**PHENOTYPIC AND GENOTYPIC IDENTIFICATION OF SALMONELLA USING ANTISENSE PEPTIDE NUCLEIC ACID PROBE - MULTITYPING.**

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**Background**

Development of novel strategies for rapid and specific identification of bacteria pathogens is urgently needed to aid successful medical and epidemiological interventions in disease outbreaks.

**Objectives**

In this study we evaluated the feasibility of altering growth phenotypes by silencing specific essential genes with antisense probes as a rapid bacterial diagnostic tool.

**Methods**

Salmonella Typhimurium and *Escherichia coli* strains were treated with antisense Peptide Nucleic Acid (PNA) probe targeting *Salmonella ftsZ* at concentrations 0-3.0µM. Cultures were grown in non-selective Muller Hinton broth (MHB), selective Rappaport-Vassiliadis Soya Peptone (RVS), and modified Rappaport-Vassiliadis Soya Peptone-low salt (mRVSS) and cell morphologies were examined by microscopy.

**Results**

If *ftsZ* silencing is successful, cells are expected to elongate, and we here observed that treatment with the Salmonella anti-ftsZ PNA treatment elongated Salmonella grown in MHB and modified RVS low salt (mRVSS), but not in RVS. In contrast, *E. coli* did not elongate when treated under the same conditions. Likewise, untreated *Salmonella cells* did not elongate. Also, modified RVS low salt media retained Salmonella selectivity and enrichment qualities.

**Conclusions**

Anti-ftsZ PNA treatment can provide both phenotypic and genotypic identification of Salmonella, and the method can be combined with selective growth conditions. We are further testing this method using artificially contaminated water and milk.

## FEMS-2611

### New diagnostic approaches

#### DEVELOPMENT OF A QPCR TO DETECT PSEUDOMONAS AERUGINOSA IN CULTURE-NEGATIVE SAMPLES FROM CYSTIC FIBROSIS PATIENTS AND MONITOR THE EFFECTIVENESS OF ANTIBIOTIC TREATMENT

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### Background

Early detection of *Pseudomonas aeruginosa* in cystic fibrosis (CF) samples is pivotal for disease eradication by antibiotic treatment. However, standard cultural methods are hampered by slow growth and alginate overproduction of mucoid variants, and possibly by viable but non-culturable state induction.

### Objectives

This work aimed at developing a qPCR for improved *P. aeruginosa* infection diagnosis in sputum from CF patients and assessment of the effectiveness of antibiotic treatment.

### Methods

Thirty-three sputum samples that were negative by culture assays were collected from 28 CF patients. DNA was extracted by the QIAamp DNA kit after centrifugation to remove free DNA. Real-time PCR assays targeting the species-specific gene *ecfX* were performed.

### Conclusions

Six/33 samples were positive for *P. aeruginosa* by qualitative Real-time PCR (LOD: 60 cells/ml) and were analyzed by qPCR (LOQ:  $3 \times 10^2$  cells/ml). *P. aeruginosa* abundance exceeded the LOQ in three samples, which carried  $1 \times 10^3$ ,  $1.6 \times 10^4$  and  $2.8 \times 10^6$  cells/ml, respectively. Three successive samples from a culture-negative, symptomatic patient, showed bacterial counts <LOQ (May 7);  $1 \times 10^3$  (still culture-negative but now receiving inhaled antibiotic therapy; May 11), and <LOQ (at the end

of antibiotic therapy; May 28). Four months later the patient was positive by cultural assays.

The matching qPCR and clinical results highlight the value of molecular assays in *P. aeruginosa* detection and quantification in CF. Dead cells cannot account for the whole difference found between PCR and culture data. Dormant forms in the pulmonary biofilm, coupled with the greater sensitivity of PCR, are likely to account for a large part of this difference.

**FEMS-2133**

**New diagnostic approaches**

**AMPEROMETRIC DETECTION OF B-LACTAMASE ACTIVITY AND ITS APPLICATION TO THE IDENTIFICATION OF EXTENDED SPECTRUM B-LACTAMASE PRODUCING STRAINS IN BLOOD SAMPLES**

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**Background**

With the ever-expanding role of extended-spectrum  $\beta$ -lactamase (ESBL) -harboring *Enterobacteraceae* in causing serious infections, rapid diagnostic assays of such resistant organisms are highly desired to improve patient screening and hospital infection control practices as well as to reduce inappropriate antibiotic use.

**Objectives**

To reach this goal, we developed an amperometric detection of the  $\beta$ -lactamase activity in blood culture samples using disposable carbon screen-printed sensors in the presence of a well-chosen cephalosporin substrate.

**Methods**

Using an ESBL enzyme isolated from a clinical strain, we showed that the intensity of a specific anodic peak current resulting from the catalytic hydrolysis of the  $\beta$ -lactam ring was proportional to the amount of ESBL. A novel susceptibility assay for the rapid and specific identification of ESBL-producing bacteria in blood culture samples was then proposed. This assay was based on a two-step protocol performed within 2 hours : (1) subculturing of blood culture samples in the presence or absence of cefotaxime and/or the potassium clavulanate (ESBL inhibitor) for a few hours followed by, (2) incubation of the subculture filtrates with the redox substrate which hydrolysis was monitored by amperometry. This assay allowed the reliable and specific identification of various types of  $\beta$ -lactamases-producing bacterial species (including ESBLs, penicillinases, cephalosporinases) in blood cultures.

**Conclusions**

Owing to its low cost, portability, simplicity and its ability to perform measurements in turbid media, the electrochemical approach, which did not require prior strain isolation, holds great promise for the rapid screening of  $\beta$ -lactamases in clinical and other complex samples.

FEMS-2135

New diagnostic approaches

## RAPID AMPEROMETRIC DETECTION OF ESCHERICHIA COLI IN WASTEWATER BY MEASURING B-D GLUCURONIDASE ACTIVITY WITH DISPOSABLE CARBON SENSORS

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### Background

Methods based on the direct measurement of  $\beta$ -D-glucuronidase (GLUase) activity in the presence of the 4-methylumbelliferyl- $\beta$ -D-glucuronide substrate are well-established to specifically monitor *Escherichia coli* (*E.coli*) in water samples without any cultivation step. However, because of the interferences from auto-fluorescence or light-quenching particle in complex samples, the fluorescence detection is often not suitable for the analysis of environmental samples.

### Objectives

To overcome this drawback, the amperometric quantification of GLUase activity was investigated and applied to the rapid and specific detection of *E. coli* in wastewater samples.

### Methods

For this purpose, the *p*-aminophenyl  $\beta$ -D-glucopyranoside (PAPG) was selected as electrochemical substrate for GLUase measurement and the *p*-aminophenol (PAP) released during the enzymatic hydrolysis was monitored by cyclic voltammetry with disposable carbon screen-printed sensors. The intensity of the measured anodic peak current was proportional to the amount of GLUase, thus providing a measurement of the number of *E.coli* in the sample (using a calibration curve).

Following an optimized protocol, we were able to detect *E. coli* cells in the range of  $5 \times 10^4$  to  $10^8$  per filter within 2 h. The amperometric assay was applied to the determination of fecal contamination in raw and treated wastewater samples and it successfully compared with conventional bacterial plating methods and *uidA* gene detection by quantitative PCR.

### Conclusions

Owing to its ability to perform cell density measurements in turbid media, the GLUase amperometric method is a reliable tool for the rapid and decentralized quantification of viable but also non-culturable *E.coli* in complex environmental samples.

## **FEMS-1325**

### **New diagnostic approaches**

#### **RAPID DETECTION OF SALMONELLA SPP. USING AN ISOTHERMAL AMPLIFICATION BASED SYSTEM, DNABLE**

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#### **Background**

The rapid, cost-effective, and robust detection of pathogens continues to be a major focus for the livestock industry. While PCR has filled the niche for a highly sensitive detection, PCR inherently burdens the end user with cumbersome sample preparation, complex and closed instrumentation with long time-to-result (TTR) (1.5-3h post-enrichment, excluding sample preparation). Other DNA amplification methods have shortened the TTR but have not been able to provide a robust and reliable alternative to PCR. The need for improved testing solutions continues to grow with growing intricacies of production logistics.

#### **Objectives**

Here we present an isothermal DNA amplification tool called DNABLE for *Salmonella* spp. detection. This system provides a distinctive advantage over other technologies as it combines high specificity and sensitivity with minimal sample preparation leading to a significantly reduced TTR (30-40 min post-enrichment, including sample preparation).

#### **Methods**

*Salmonella* detection was demonstrated with an analytical sensitivity of <50cp of genomic DNA per reaction or <10 cells post-enrichment in multiple test matrices. Inclusivity and exclusivity data indicate 100% accuracy across 34 *Salmonella* serotypes and 29 non-*Salmonella* tested strains.

#### **Conclusions**

DNABLE requires minimal sample preparation due to its robust chemistry and is compatible with a range of detection equipments. *Salmonella* spp. detection system also includes an internal amplification control, *Salmonella* selective media supplement that improves current enrichment, and a user-friendly sample preparation kit. Results are provided by a portable and simple DNABLE reader or user provided thermocycler. The inherent robustness, quick TTR, and broad range instrument compatibility support use of DNABLE for *Salmonella* detection.



**FEMS-2989**

**New diagnostic approaches**

**FABRICATION OF DNA ELECTROCHEMICAL BIOSENSOR BASED ON CARBON NANOTUBES FOR DETECTION OF FECAL CONTAMINATED WATER WITH ENTEROCOCCUS FAECALIS**

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**Background**

Fecal pollution of water can lead to health problems due to presence of infectious microorganisms especially *Enterococcus faecalis* (*E. faecalis*) which may be derived from human sewage or animal sources. Hence, detection of these microorganisms is of much interest using different methods. Electrochemical DNA biosensors, which employ an immobilized DNA as the biological recognition element, are currently under intense investigation in detection of several microorganisms relying on the conversion of the base-pair recognition event into a useful electrical signal.

**Objectives**

The aim of this study was to demonstrate a rapid and inexpensive method for accurately detection of *E. faecalis* in contaminated water via fabrication of a specific and selective DNA electrochemical biosensor.

**Methods**

A modified DNA oligonucleotide with 5' amino modifier C12 was designed based on a house keeping gene glucose 6-phosphate dehydrogenase of *E. faecalis*. The multi walled carbon nanotubes (MWCNT) were allowed to form amide bond between their carboxylic acid groups and amino groups of oligonucleotides. Hybridization was performed by incubating the DNA/self-assembled MWNTs with complementary DNA oligonucleotides, non-complementary DNA oligonucleotides, genomic DNA and artificial contaminated water under specific conditions for 30 min at room temperature. The hybrid-electrodes were successively transferred into hybridization indicator of methylene blue for 30 min without any potential followed by several washings and subsequently electrochemical measurements.

**Conclusions**

Such single-use electrochemical biosensors have great promise for decentralized water testing for presence of *E. faecalis* in an accurate and rapid approach.



**FEMS-1258**

**New diagnostic approaches**

## **COMPARISON OF TWO METHODS FOR DNA EXTRACTION FROM SPUTUM SAMPLES IN COPD PATIENTS**

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### **Background**

Acute exacerbations of chronic obstructive pulmonary disease (AE COPD) are predominantly caused by microbial pathogens. Molecular techniques may improve the speed and sensitivity of respiratory infections diagnostics. Currently there is limited knowledge on the best method for DNA extraction from sputum samples.

### **Objectives**

This study is a part of the European JRP HLT08 INFECT-MET. The aim was to evaluate the DNA yield of two different extraction methods, manual and automated, in treated and untreated sputum samples from COPD patients.

### **Methods**

A total of 47 good-quality sputum samples from COPD patients between March 2014 and October 2014 were included in the study. Twenty samples were left untreated and 27 were treated with NALC-NaOH. All samples were split in two aliquotes and DNA was extracted with manual (QIAamp DNA Mini Kit, Qiagen GmbH, Germany) and automated (DNA Extraction Kit, DiaSorin, Italy/NorDiag Arrow) method. Concentration of DNA was quantified (Qubit Fluorometer, Invitrogen, USA).

### **Conclusions**

Out of 47 sputum samples 41 (87,2%) had higher DNA concentration with automated extraction method. Among 20 untreated sputum samples automated method had a higher DNA yield in 14 samples by 68,1%, lower in 5 by 43,1 % and equal in 1 sample. In 27 treated sputum samples automated extraction method had a higher DNA yield in all 27 samples by 60,9%.

Automated extraction method proved to be more efficient in extracting DNA from untreated and treated sputum samples. Automated extraction method is much easier to perform and less time consuming.

**FEMS-0844**

**New diagnostic approaches**

## **DEVELOPMENT OF DUAL-FUNCTION ELISA FOR EFFECTIVE ANTIGEN AND ANTIBODY DETECTION AGAINST H7 AVIAN INFLUENZA VIRUS**

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### **Background**

Outbreaks in poultry involving influenza virus from H7 subtype have resulted in human infections, thus causing a major concern for public health, as well as for the poultry industry. Currently, no efficient rapid test is available for large-scale detection of either antigen or antibody of H7 avian influenza viruses.

### **Objectives**

In the present study, a dual function ELISA was developed for the effective detection of antigen and antibody against H7 AIVs.

### **Methods**

The test was established based on antigen-capture-ELISA and epitope blocking ELISA. The two Mabs 62 and 98 which were exploited in the assay were identified to recognize two conformational neutralizing epitopes on H7 HA1. Both of the epitopes exist in all of the human H7 strains, including the recent H7N9 strain from China and > 96.6% of avian H7 strains. The dual ELISA was able to detect all of the five H7 antigens tested without any cross reaction to other influenza subtypes. The antigen detection limit was less than 1 HA unit of H7. For antibody detection, the sensitivity and specificity of the dual ELISA was evaluated and compared to HI and microneutralization using immunized animal sera to different H7 strains and different subtypes of AIVs. Results indicated that antibodies to H7 were readily detected in immunized animal sera by the dual ELISA whereas specimens with antibodies to other AIVs yielded negative results.

### **Conclusions**

This is the first dual-function ELISA reported for either antigen or antibody detection against H7 AIVs. The assay was highly sensitive and 100% specific in both functions rendering it effective for H7 diagnosis.

## FEMS-1400

### New diagnostic approaches

#### NEOPTERIN AND SOLUBLE CD14 LEVELS IN CASES WITH UNDETERMINATE AND CONFIRMED HIV-1 INFECTIONS RELATED WITH HIV-1/2: DOES UNDETERMINATE HIV-1 PATTERN SHOW A REAL HIV-1 INFECTION?

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#### Background

HIV-1/2 testing algorithms are currently controversial. The most important problem and difficulty about this debate is undeterminate HIV-1 infections (UHIV-1). Studies for an optimised algorithm for UHIV-1 are under research.

#### Objectives

In this study, we aimed to determine neopterin and sCD-14 levels in cases with UHIV-1 and confirmed HIV-1 (CHIV-1) infections and evaluate the relationship between UHIV-1 and real HIV-1 infection and also aimed to evaluate the diagnostic values of two markers, especially in situations when PCR were not available and western blot were undeterminate after ELISA.

#### Methods

Eighty-eight cases with UHIV-1, 100 patients with CHIV-1 and 100 healthy control group (HCG) were included. Neopterin and sCD-14 levels were determined by competitive and sandwich ELISA (nmol/mL, µg/mL, quantitatively) methods, respectively. Statistical tests were performed using SPSS 21.0 and  $p < 0.05$  was considered significant.

#### Conclusions

##### Results:

Table 1. Neopterin and sCD-14 levels of UHIV-1 and CHIV-1 cases and healthy control group

Markers (n:100)	Undeterminate HIV-1 <sup>1</sup> (n:88) HCG <sup>3</sup> (n:100)	Confirmed-HIV-1 <sup>2</sup>
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Neopterin, mean (min-max)	5.73 (1.58-38.71)	15.77 (1.19-127.19)	7.95 (3.16-41.08)
sCD14, mean (min-max)	3.65 (1.86-6.17)	4.20 (1.53-7.59)	3.51 (0.97-3.97)

P value: Neopterin: 1x2; p:0.004, 1x3; p:0.754

sCD14: 1x2; p:0.034, 1x3; p:0.787

Table 2. Diagnostic test performances of neopterin and sCD14 in CHIV-1 infections

Markers	Sensitivity	Specificity	PPV	NPV	Kappa coefficient
Neopterin (%)	38	82	76	57	20
sCD14 (%)	50	100	100	67	50

As conclusion,, we couldn't suggest UHIV-1 pattern as real HIV-1 infection and we concluded that two markers were useless as surrogate markers for real HIV-1 infections.

## FEMS-1404

### New diagnostic approaches

#### NEOPTERIN AND SOLUBLE CD14 LEVELS IN CASES WITH ISOLATED ANTI-HBC PATTERN AND CHRONIC ACTIVE HEPATITIS : DOES ISOLATED ANTI-HBC PATTERN SHOW REAL HBV INFECTION?

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#### Background

Isolated Anti-HBc pattern (IAH) positivity causes diagnostic difficulty in laboratory diagnosis of HBV infections. In addition, this pattern is a big problem for clinical diagnosis and blood transfusion.

#### Objectives

In this study, we aimed to determine neopterin and sCD-14 levels in cases with IAH and chronic active hepatitis (CAH) and to evaluate the relationship between IAH and real HBV infection and also aimed to evaluate the diagnostic values of two less sophisticated and cheaper surrogate markers in situations when the molecular methods were not available.

#### Methods

IAH (n:102), KAH (n:70) and healthy control group (HCG), (n:100) cases were included in the study. Neopterin and sCD-14 levels were determined by competitive and sandwich ELISA (nmol/mL, µg/mL, quantitatively) methods, respectively. Statistical tests were performed using SPSS 21.0 and p < 0.05 was considered significant.

#### Conclusions

Table 1. Neopterin and sCD-14 levels in IAH, KAH and HCG cases .

Markers	IAH <sup>1</sup>	KAH <sup>2</sup>	HCG <sup>3</sup>

	(n:102)	(n:70)	(n:100)
Neopterin, mean (min-max) (3.16-41.08)	8.67 (3.56-67.15)	15.43 (4.75-43.27)	7.95
sCD14, mean (min-max) (0.97-3.97)	3.61 (1.82-6.17)	4.20 (1.96-6.78)	3.51

p value: Neopterin: 1x2; p:0.002, 1x3; p:0.915

sCD14 : 1x2; p:0.006, 1x3; p:0.821

Table 2. Diagnostic test performances of neopterin and sCD14 in real HBV infections

Markers coefficient	Sensitivity	Specificity	PPV	NPV	Kappa
Neopterin (%)	59	82	69	75	42
sCD14 (%)	51	90	78	73	44

As conclusion, we couldn't suggest IAH pattern as a real HBV infection and we concluded that two markers were useless as surrogate markers for the diagnosis of HBV infections.



**FEMS-2559**

**New diagnostic approaches**

**DETECTION OF THE OOMYCETE PYTHIUM INSIDIOSUM BY REAL-TIME PCR OF THE EXO-1,3-B-GLUCANASE GENE**

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**Background**

Pythiosis is a life-threatening infectious disease caused by the pathogenic oomycete *Pythium insidiosum*. The disease has high rates of morbidity and mortality. Timely diagnosis permits prompt treatment and a better prognosis. *PinsEXO1*, encoding an exo-1,3- $\beta$ -glucanase, is a novel and efficient target for identification of *P. insidiosum* by conventional PCR.

**Objectives**

In this study, we aimed to develop a real-time PCR approach targeting *PinsEXO1* and compare its performance to conventional PCR for the detection of *P. insidiosum*.

**Methods**

Genomic DNA samples were prepared from 35 culture-proven *P. insidiosum* isolates and 58 culture-proven fungi (served as the control), for PCR analyses. Both conventional and real-time PCR assays were positive for all *P. insidiosum* strains tested, while all control fungi were negative. Turnaround time for conventional PCR was 4 hr, while that of real-time PCR was 1.5 hr. The minimum DNA template required for successful PCR amplification by conventional and real-time PCR were 1 ng and  $1 \times 10^{-4}$  ng, respectively.

**Conclusions**

The real-time assay retained high detection sensitivity and specificity. It showed a substantially improved analytical sensitivity and turnaround time that could improve diagnoses of pythiosis.

**FEMS-2304**

**New diagnostic approaches**

**DEVELOPMENT OF A NOVEL MALARIA ANTIBODY ASSAY UTILIZING ANTIGENS FROM ALL 5 HUMAN PATHOGENIC PLASMODIUM SPECIES**

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**Background**

The proper diagnosis of Malaria disease is essential to provide early treatment and improve the prognosis of patients. Transfusion-transmitted Malaria is rare, but it may produce severe problems in the safety of blood Transfusion and blood related products due to the lack of reliable procedure to evaluate donors potentially exposed to malaria.

**Objectives**

Microscopy, still considered the gold standard for diagnosing malaria. It is time consuming and requires trained expertise. ELISAs are known to be ideal for high throughput screening with high sensitivity and specificity, but it also requires trained personal and an equipped laboratory. Line Blots are often used as confirmatory tests since they provide high sensitivity and specificity. There is nearly no lab equipment needed to perform this kind of assay. In addition, blots can also be used in automated processes for high throughput screening.

**Methods**

Here we show an improved diagnostic performance of the new antibody detection Systems (ELISA and Lineblot) utilizing early and late antigens of all 5 human pathogenic Plasmodium species (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*) compared to test systems only relying on antigens derived from one or two Plasmodium species. Assays with a limited number of antigens often fail to detect antibodies from certain regions of the world. For evaluation purpose, we collected samples from all over the world, including samples from newborns.

**Conclusions**

We evaluated the performance of ELISA and Lineblot directly in endemic countries with samples of patients who presented symptoms akin to malaria infection in local hospitals.



**FEMS-1337**

**New diagnostic approaches**

**INVESTIGATION OF ORTHOPAEDIC IMPLANT ASSOCIATED INFECTIONS:  
ALTERNATIVE APPROACHES TO LABORATORY PROCESSING AND CULTURE**

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*y*<sup>1</sup>

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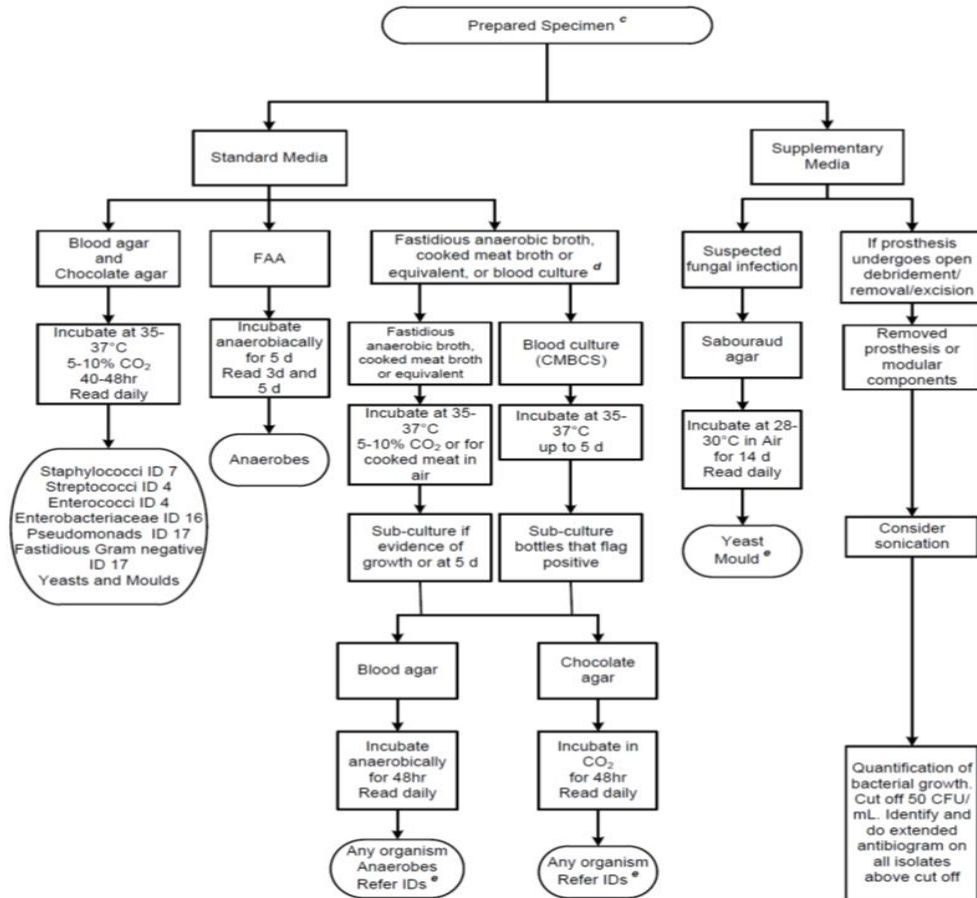
**Background**

Since its introduction in the 1960s, joint replacement (arthroplasty) has become a common procedure. Around 15% of revisions are due to 'septic' loosening caused by biofilm formation on the implant. Infection rates are now much lower; however, there is still a risk associated with each procedure. UK SMI B44: is a diagnostic tool aimed at practising professionals in the field of microbiology. It describes and recommends methods for the microbiological investigation of orthopaedic implant associated infection.

**Objectives**

The aim of this poster is raise awareness of the various methods of sample preparation (including the use of glass beads and sonication) and culture. Timely diagnosis and treatment can help reduce the likelihood of systemic dissemination of infection. Therefore, in most cases, this UK SMI recommends up to 5 days culture using either cooked meat broth or continuous monitoring blood culture system

methods.



## Methods

Produced by the Standards Unit, UK SMIs are developed, reviewed and updated through a wide consultation process with users and other stakeholders. The process follows the AGREE tool and resulting documents reflect best evidence based practice. Where evidence is not available, the documents are based on national working group consensus decisions.

## Conclusions

B44 provides guidance on the best minimum practice when investigating orthopaedic implant associated infection. It is a useful resource for laboratories which aims to help drive pathology modernization by recommending alternative technologies for timely laboratory diagnosis of infection.



## FEMS-2740

### New diagnostic approaches

#### MASS SPECTROMETRY PROTEOTYPING FOR THE DETECTION, CHARACTERISATION AND IDENTIFICATION OF INFECTIOUS BACTERIA

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### Background

Diagnosing infectious diseases requires a range of methodologies for the comprehensive detection, characterisation and identification of infectious bacteria. Proteomics-based approaches represent an alternative to traditional methods of characterising microorganisms, elucidating expressed biomarkers of genome information, applied to 'proteotyping' microorganisms at all taxonomic levels.

### Objectives

Apply state-of-the-art and novel techniques of tandem mass spectrometry (MS) analyses of expressed proteins for species- and strain-level identification and detection of antibiotic resistance and virulence, with the focus on optimizing MS-based proteomics analyses for 'proteotyping' infectious bacteria.

### Methods

Intact bacteria or cell fractions were immobilised, via membrane-gold interactions within a flow-cell. Within the flow-cell, proteins were subjected to enzymatic digestion to generate peptides, which were identified, using LC-MS/MS. Following database matching, peptides were used for bacterial species-level identification, and for detection of antibiotic resistance and sub-species typing. To demonstrate proteotyping capability and differentiate closely related species, mixtures of different *Streptococcus* spp. were analysed. Results of *Streptococcus* spp. identifications from clinical samples were confirmed by standard microbiology, including cultivation of bacteria in selective media, PCR, DNA sequencing and MALDI-TOF MS analyses.

### Conclusions

Proteotyping of infectious bacteria, using LC-MS/MS enabled the differentiation and identification of *Streptococcus* spp. by ranking identified expressed proteins, according to the number of peptide matches to genome sequence information available in databases, and was applied to detection in clinical samples. The methods

have been shown to enable reproducible, sensitive, rapid and cost-effective typing of bacteria for diagnostics of infectious diseases.



## **FEMS-2765**

### **New diagnostic approaches**

#### **APPLICATION OF MOLECULAR TECHNIQUES TO THE RAPID DETECTION AND CONFIRMATION OF HIV**

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#### **Background**

Western Blot (WB) is the gold standard as HIV-1 confirmatory test, which has high cost and complexity in processing, our setting send the reactive samples to a national reference center for confirmation and reporting of results takes at least 15 days.

#### **Objectives**

The aim of this work was to standardize a technique multiplex PCR as a complementary test for the detection and confirmation of HIV-1 infection in Oaxaca, Mexico.

#### **Methods**

Standardization of the multiplex PCR was perform using primers designed to amplify the *gag*, *pol* and *env* genes of M, N, O groups and other specific to Subtype B. For the standardization, we use DNA of three patients that was previously characterized as HIV positive and samples sequenced for HIV-1 (*pol*, *gag*, *env*). We used this multiplex PCR in DNA samples from PMBC of 600 patients. All patients were screening by HIV rapid test. To confirm HIV infection, both WB and multiplex PCR were perform.

#### **Conclusions**

We obtain concordant results with multiplex PCR and those of WB. The implementation of a multiplex PCR as a supplemental test for confirmation of HIV-1 infection is reliable and safe. Its implementation has the possibility of decreasing the time of detection and reporting of confirmatory results to a day, which is essential, for example, in cases of pregnant women the confirmation or discard of HIV must be timely, with the aim to start prophylaxis on time.

**Acknowledgements:** This work is found by Fondo Mixto CONACYT-Gobierno del Estado de Oaxaca, FOMIX 193298



**FEMS-0969**

**New diagnostic approaches**

**INFLUENCE OF STORAGE CONDITIONS ON THE SPECTRAL STABILITY OF BACTERIAL SUSPENSIONS IDENTIFIED BY MALDI BIOTYPER**

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**Background**

Although direct smear technique is an excellent approach for the preparation of samples for MALDI-TOF MS identification, the usefulness of this approach is limited to BSL-1/BSL-2 bacteria, it is not recommended for MSP creation and it requires fresh material for an optimal performance. Formic acid extraction is proposed as a more precise method for MALDI Biotyper identification that includes a step in which bacteria are suspended in 70% ethanol which could potentially be used for the storage of bacterial suspensions in their optimal growth phase. However, there is a lack of information regarding the impact of storage of bacterial suspensions in ethanol on the the reliability of the MALDI-Biotyper identification.

**Objectives**

A study was carried out aiming to assess the influence of time (90 days) and temperature (room temperature and 4°C) on the identification by MALDI Biotyper.

**Methods**

Two bacterial suspensions (*E. coli* and *S. aureus*) stored in ethanol 70% were included. Half of the aliquots (n=27) were conserved at room temperature and the other half at refrigeration until the day of analysis (0, 5, 8, 12, 19, 26, 42, 71 and 90 days).

**Conclusions**

Our data suggest that *E. coli* and *S. aureus* ethanol suspensions can be stored up to 90 days for MALDI Biotyper identification in laboratory conditions. Although it has to be validated, it may apply to other bacteria and could greatly facilitate laboratory work-flow and shipment of bacterial suspensions

**FEMS-0716**

**New diagnostic approaches**

**IDENTIFICATION OF H. PYLORI EPITOPES RESPONSIBLE FOR HOST IMMUNO-RESPONSE MODULATION THROUGH ORF-FILTERED PHAGE DISPLAY LIBRARIES AND INTERACTOME-SEQUENCING**

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**Background**

To elucidate the molecular mechanisms involved in persistency/latency of the *H.pylori* infection or in its progression towards serious diseases, it is necessary to analyze the host pathogen interaction in vivo. The circulating antibody repertoire represents an important source of diagnostic information, serving as biomarker to provide a 'disease signature'.

**Objectives**

The aim of this work is the identification of *H.pylori* epitopes responsible for host immunoresponse modulation through a discovery-driven approach that couples 'phage display' and deep sequencing.

**Methods**

We used an approach for identifying novel antigens by screening gDNA libraries created from the pathogen genome, directly with sera from infected patients. Three phage display libraries from three *H.pylori* strains (HP26695, HPB128, HPSS1) have been constructed by using  $\beta$ -lactamase ORF selection vectors (Di Niro et al., 2010). Genomic DNA was sonicated, fragments cloned into the filtering vector, after transformation libraries of  $1 \times 10^6$  clones were obtained and sequenced by 454 technology.

**Conclusions**

More than 93% of HP CDSs were represented in the phage genomic library therefore being representative of the whole *H.pylori* antigenic ORFeome. Putative antigens were selected from libraries using sera from patients affected by *H. pylori* presenting increasing degrees of infection: i) autoimmune gastritis and pernicious anemia; ii) gastric adenocarcinoma; iii) MALT lymphoma. The results show that the diversity of the libraries obtained after selection is significantly reduced. Furthermore, individual ranks, for each infection condition, have been compared highlighting the pattern of putative antigens, shared by all the conditions, and some that can distinguish the different stages of infection.

**FEMS-0652**

**New diagnostic approaches**

**DETECTION OF CRYPTOSPORIDIUM OOCYST RNA BY SURFACTANT EXTRACTION TREATMENT AND RT-PCR: INHIBITION OF REVERSE TRANSCRIPTION BY SDS AND ITS SUPPRESSION USING NONIONIC SURFACTANTS**

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**Background**

*Cryptosporidium* causes the symptoms of cryptosporidiosis including watery diarrhea, vomiting and fever. *Cryptosporidium* oocysts possess a robust wall, which is extremely resistant to chlorine used for potable water disinfection. Successful DNA detection from the robust oocyst usually requires complicated procedures including DNA extraction such as freeze-thaw cycling and enzyme treatment. Therefore, we previously developed a surfactant extraction treatment (SET) method for extracting DNA from the oocysts with only sodium dodecyl sulfate (SDS).

**Objectives**

We examined the inhibition of reverse transcription-PCR (RT-PCR) by SDS and its suppression using four kinds of nonionic surfactants in order to develop a new method for detecting 18S rRNA from the oocyst by SET and RT-PCR.

**Methods**

The template DNA/RNA was amplified in the presence of SDS and/or the nonionic surfactants; Tween 20, Tween 80, Triton X-100, Triton X-114 by RT-PCR to evaluate the inhibition of reverse transcriptase and DNA polymerase. The oocysts were incubated in a 0.1% SDS solution to extract DNA/RNA.

**Conclusions**

DNA/RNA amplification was inhibited at 0.01% SDS but its inhibition was suppressed by adding any of the nonionic surfactants at 5%. There was a difference in the suppression efficacy of the nonionic surfactants in tests that used 0.1% SDS. 18S rRNA molecules are present in high copy numbers in viable cells, which increases detection sensitivity. Therefore, we succeeded to detect 18S rRNA equivalent to 10<sup>-4</sup> oocysts by SET and RT-PCR. This work was supported in part by JSPS KAKENHI Grant Number 25420559 and the Kurita Water and Environment Foundation.

**FEMS-2462**

**New diagnostic approaches**

**NEW DIAGNOSTIC APPROACHES IN INFECTION DETECTION BASED ON THE IMMUNE SYSTEM DERIVED ENZYMES IN WOUND FLUID**

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**Background**

Quick and proper wound diagnostics could avoid wound infections of currently 5-10% of post-surgical - and 25% of chronic wound patients. Apart from standard methods, this study focusses on the wound fluid, harboring enzymes of the human immune system and reflecting a critical wound status upon increasing activity.

**Objectives**

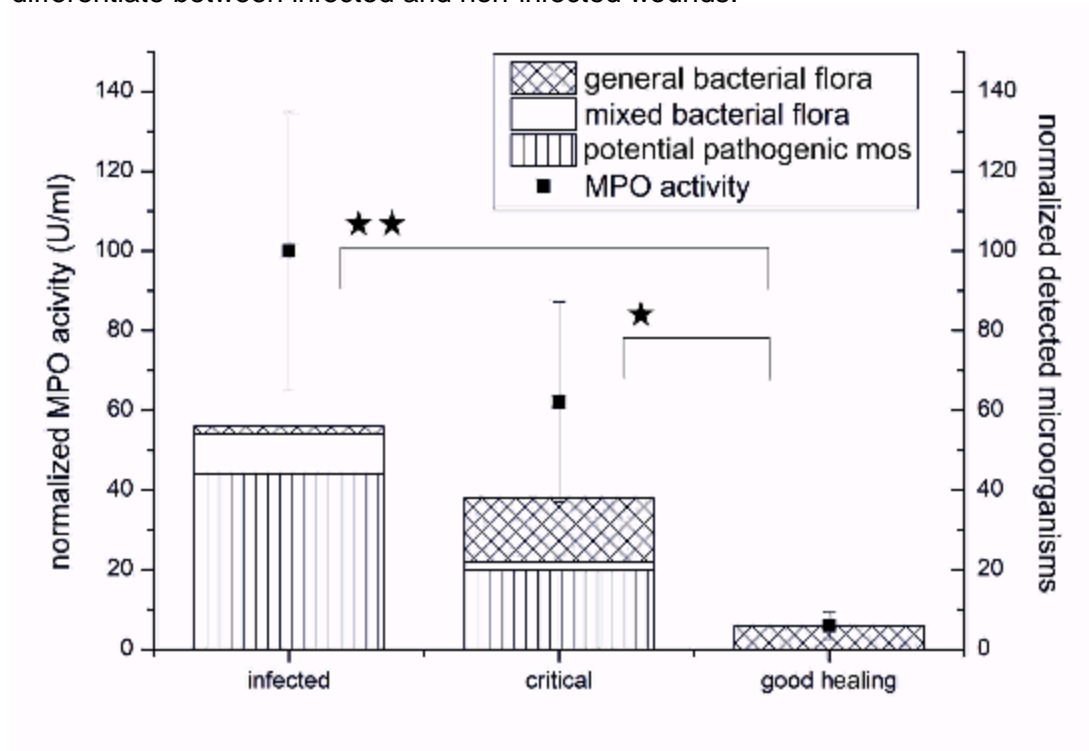
The determination of the enzyme levels of the neutrophil derived enzymes human neutrophil elastase (HNE), myeloperoxidase (MPO) and lysozyme (LYS) was assessed and correlated to the bacterial burden of the wounds [1]. New diagnostic approaches were based on these enzymes and include electrochemical and optical sensor systems for fast diagnosis of infections in chronic wounds [2].

**Methods**

The enzyme activities of HNE, LYS and MPO were directly monitored in wound fluid of affected patients via biochemical investigations. Both electrochemical sensors such as measuring the hydrogen peroxide consumption by MPO as current decrease and lateral flow devices based on release of dyes were developed.

**Conclusions**

Determination of the enzyme activities showed more than 6 to 10 fold higher substrate conversion in infected wound fluids when compared to non-infected wound fluids and clearly correlated with the elevated bacterial burden (Fig1). Consequently, both the electrochemical sensor as well as lateral flow devices were able to differentiate between infected and non-infected wounds.



- [1] A. Hasmann, U. Gewessler, et al., *Exp Dermatol.* **(2011)** 20:508–13.
- [2] M. Hajnsek, D. Schiffer, et al., *Sensor ActuatB-Chem.* **(2015)** 209:265–274.



## FEMS-3108

### New diagnostic approaches

#### MOLECULAR SURVEILLANCE ON NASOPHARYNGEAL CARRIAGE OF STREPTOCOCCUS PNEUMONIAE IN CHILDREN VACCINATED WITH CONJUGATED POLYSACCHARIDE PNEUMOCOCCAL VACCINES

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#### Background

As a commensal of the upper respiratory tract, *Streptococcus pneumoniae* is a potential pathogen causing respiratory and invasive diseases. Following implementation of pneumococcal conjugate vaccination (PCV) for infants, surveillance studies have proven essential for monitoring direct (carriage of serotypes targeted by vaccine, VTs) and indirect effects (changes in carriage of non-vaccine serotypes, NVTs).

#### Objectives

To compare the detection of pneumococcal carriage and serotypes in a unique study setting using both conventional culture and molecular methods, in nasopharyngeal samples from healthy PCV-vaccinated infants in two large, cross-sectional surveillance studies on PCV-effects in the Netherlands.

#### Methods

Nasopharyngeal samples were collected from 1182 11- and 24-month old children (n=591 each) during autumn/winter 2010/11 (n=584) and 2012/2013 (n=598). Following conventional culture on plates selective for *S. pneumoniae*, DNA extracted from all bacterial growth was tested by quantitative-PCR (qPCR) for the presence of pneumococci and a panel of serotypes, including serotypes targeted by the thirteen-valent PCV (PCV13).

#### Conclusions

There was a correlation (Spearman's rho=0.980;  $p<0.001$ ) between the frequency of serotypes detected using qPCR and prevalence according to conventional culture. Moreover, we observed a trend ( $p=0.056$ ) for an underestimation of carriage detection for serotypes not targeted by PCV7 or PCV10 (in use in the Netherlands) compared to PCV10-serotypes when detected using the culture-method alone. We found no evidence of a hidden circulation of serotypes rarely detected by culture or those targeted by vaccination. This suggests that surveillances based on the culture method alone do not underestimate carriage of VTs in immunised children.



**FEMS-2235**

**Secondary metabolites, metabolomics**

**INVESTIGATION OF MICROBIAL INTERACTIONS IN MINIATURIZED FUNGAL CO-CULTURES BY MS-BASED METABOLOMICS**

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**Background**

In the field of natural product research, finding new sources of bioactive compounds is of primary importance. In this respect, microorganisms have provided a large number of biologically active molecules.

**Objectives**

Recently, the use of fungal co-cultures for the induction of new natural products has emerged as a promising field in drug discovery.

For the success of such studies, a key element is the development of a co-culture methodology that provides high reproducibility of metabolite induction patterns and that is compatible with high throughput analytical procedures.

**Methods**

To tackle this issue, a method based on 12-well-plate miniaturized Petri dishes compatible with high throughput UHPLC-TOF-MS metabolomics [3] has been developed. This strategy was used to screen for metabolite induction in co-cultures of various fungal species. This approach provided a satisfactory reproducibility and was used for the identification of induced biomarkers.

**Conclusions**

This study demonstrates the consistent induction of new metabolites through co-culture. Moreover, the developed strategy is generic and can be applied to other types of microorganisms that can grow on solid media and that are part of the myco- or microbiome. This 12 well-plate approach and the adapted data mining strategy were validated by the untargeted metabolomic study of a model co-culture (*Eutypa lata* versus *Botryosphaeria obtusa* responsible for confrontation zone lines in *Vitis* wood). This procedure is currently used for screening novel metabolite induction in various fungal co-cultures.

**FEMS-2656**

**Secondary metabolites, metabolomics**

## **EXPANDING THE DIVERSITY OF THE CYCLODIPEPTIDE SYNTHASE FAMILY**

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### **Background**

Cyclodipeptide synthases (CDPSs) constitute a novel family of enzymes that use charged tRNAs to synthesize cyclodipeptides in biosynthetic pathways dedicated to the synthesis of diketopiperazines, a large class of secondary metabolites with noteworthy biological activities. To date, 11 CDPSs have been biochemically characterized, showing the incorporation of hydrophobic amino acids in cyclodipeptides (essentially Phe, Leu, Tyr and Trp). Biochemical and structural characterizations of three CDPSs identified catalytic residues and allowed to propose a ping-pong mechanism for cyclodipeptide formation. However, bioinformatic searches in databases identified in microbial genomes numerous putative CDPS whose activities are not predictable. Furthermore, some of these CDPSs showed differences in predicted catalytic residues.

### **Objectives**

Our objective was to provide a better characterization of the CDPS family of enzymes notably by determining the cyclodipeptide-synthesizing activity of a large set of unrelated putative CDPSs identified in databases.

### **Methods**

We used bioinformatic tools to select the CDPS candidates for biochemical characterization and to analyze their amino acid sequences. As previous work showed the recovery of cyclodipeptides in culture supernatants upon CDPS expression in *Escherichia coli*, CDPSs were produced in this host and cyclodipeptides were searched in culture supernatants by HPLC coupled to mass spectrometry.

### **Conclusions**

The determination of the activities of 41 novel CDPSs enlarged the chemical diversity synthesized by these enzymes, as they were shown to incorporate 17 of the 20 proteinogenic amino acids. CDPSs can be classified into several phylogenetically distinct subfamilies characterized by specific functional subsequence signatures, suggesting differences in catalysis by members of the two subfamilies.

**FEMS-0908**

**Secondary metabolites, metabolomics**

## **IDENTIFICATION OF ANTIMICROBIAL LIPOPEPTIDES OF BACILLUS STRAINS OBTAINED BY DIFFERENT WAYS OF EXTRACTION USING MALDI-TOF MASS SPECTROMETRY**

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### **Background**

*Bacillus* cyclic lipopeptides of surfactin, iturin, fengycin, and recently discovered kurstakin families have well-recognized potential in biotechnology and biopharmacies. Analytical method such as matrix-assisted laser desorption ionization-time of flight mass (MALDI-TOF) spectrometry proved very effective for detection and identification of various antimicrobial substances.

### **Objectives**

The present study was aimed at investigating how different mode of extraction of crude lipopeptides produced by different *Bacillus* strains influence the amount of crude lipopeptide and highlights the best extraction mode for further use.

### **Methods**

Five proven lipopeptide producers were grown under previously determined conditions and consequently extracts were obtained by ethyl acetate extraction and by combination of acid precipitation and methanol extraction. Also, cell-free supernatants in the form of aqueous extracts were examined. Extracts were subjected to MALDI-TOF-MS analysis in the acquisition mass range 800-1700 Da.

### **Conclusions**

Mass spectra obtained from all extracts tested, showed clearly three peak clusters corresponding to different lipopeptide families. Cluster of peaks, corresponding to iturin and surfactin families, with similar intensities and points to the dominant presence of these compounds in all strains and extracts, was observed. However, for peaks within the mass range of 1450-1540, corresponding to fengycin family, the best detection was achieved for ethyl acetate extracts. The peaks in the mass range of kurstakin family, for most cases, were more pronounced in the aqueous extract, while the lowest detection was obtained for methanolic extraction. In conclusion, the most efficient was ethyl acetate extraction, which gives the most prominent peaks together with the aqueous extract as a positive control.

**FEMS-2622**

**Secondary metabolites, metabolomics**

## **BIOSYNTHESIS AND DEGRADATION OF KLEBSIELLA OXYTOCA ENTEROTOXIN TILIVALLINE**

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### **Background**

Antibiotic-associated hemorrhagic colitis (AAHC) develops during antibiotic-driven intestinal dysbiosis and is caused by production of the bacterial pyrrolobenzodiazepine tilivalline.

### **Objectives**

To understand the function of this secondary metabolite during health and disease we study the regulation of tilivalline biosynthesis and degradation.

### **Methods**

Genes involved in tilivalline biosynthesis were identified via transposon mutagenesis of the clinical isolate AHC-6. Genes encoding a 3-deoxy-D-arabino-heptulosonate-7-phosphate (DAHP) synthase (*aroX*), a dehydroquinate-synthase (*aroB*) and two non-ribosomal peptide synthases (*npsA* and *npsB*) were identified as essential genes for tilivalline production. Adjacent genes *npsA*, *npsB* and *aroX* are located on a pathogenicity island present in all toxin positive *Klebsiella* isolates.

Under laboratory conditions tilivalline is detected in conditioned medium of bacterial cultures. Cytotoxicity towards human epithelial HeLa cells appears at the end of the exponential phase (8 h), reaches a maximum during stationary phase and decreases after 40 h to undetectable levels at 48 h. The loss of cytotoxicity can indicate a growth-dependent regulation of biosynthesis and/or inactivation of tilivalline. Emergence of degradation products in tilivalline-depleted supernatant was analyzed by HPLC-MS and NMR. Regulation of tilivalline biosynthesis genes in vitro was monitored via qRT-PCR.

### **Conclusions**

We find that transcription of *npsA* and *npsB* is regulated in a growth dependent

manner. Current studies focus on effects of host and microbial factors on tilivalline biosynthesis and cytotoxicity.



**FEMS-1095**

**Secondary metabolites, metabolomics**

**BURKHOLDERIA GENOME MINING FOR NONRIBOSOMAL PEPTIDE SYNTHETASES REVEALS A GREAT POTENTIAL FOR NOVEL LIPOPEPTIDES SYNTHESIS**

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**Background**

Many of natural compounds produced by microorganisms, are synthesized by non ribosomal peptides synthetases (NRPS), working as assembly lines. Among them, lipopeptides as those produced by *Bacillus* and *Pseudomonas*, may play an important role in sustainable agriculture, used as biopesticides to kill plant pathogens.

**Objectives**

As lipopeptides are synthesized by NRPS, we have decided to screen and identify *in silico* all NRPS gene clusters of *burkholderia*, especially those producing siderophores and lipopeptides, potentially implicated in biocontrol of phytopathogens.

**Methods**

We performed *in silico* detection and analyses of NRPS genes present in 48 gapless complete genomes of *Burkholderia* available in the NCBI, by following Florine, a workflow we have developed especially with this aim.

**Conclusions**

Genome analyses revealed 161 clusters containing NRPSs, with the potential to synthesize at least 11 novel products. Although most strains produce the main siderophore ornibactin or malleobactin, a cluster corresponding to a new siderophore, called phymabactin, was identified in *Burkholderia phymatum* STM815 and the cluster for cepaciachelin biosynthesis was, for the first time, identified in *Burkholderia ambifaria* AMMD. Elsewhere, the cluster for the antifungal burkholdin was detected in both the genomes of *B. ambifaria* AMMD and *Burkholderia* sp. KJ006. We also

identified a novel lipopeptide called burkhomycin specifically produced by *Burkholderia pseudomallei* strains. This study revealed the significance of the genus *Burkholderia* as a promising source of bioactive compounds. It also gave new insights on the non-ribosomal synthesis exemplified by the identification of dual C/E domains in lipopeptide NRPSs, as frequently found in *Pseudomonas* strains.

**FEMS-1299**

**Secondary metabolites, metabolomics**

**BACTERIAL TRIGONELLINE METABOLISM, FROM THE GENES TO THE METABOLITES: A STEP FORWARD INTO THE METABOLIC LANDSCAPE OF BACTERIA**

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**Background**

Trigonelline (N-methylnicotinate), a pyridine alkaloid, is produced by plants to resist draught stress. Its catabolism by soil bacteria is presumed to proceed through methylamine and succinate but no pathway is described until now.

**Objectives**

Our model organism is *Acinetobacter baylyi* ADP1, a strictly aerobic, nutritionally versatile soil bacterium which is easily amenable to genetic manipulations. Biological tools and ressources have been developed in the laboratory to enable the integrative analysis of its metabolism. Since it grows well on trigonelline, we wanted to elucidate this pathway at the finest level.

**Methods**

By comparative genomics, we could identify a conserved cluster of 10 genes involved in trigonelline breakdown. We dissected the pathway by combining bacterial physiology with mutant strains, classical enzymology with recombinant enzymes and untargeted LC/MS based metabolomic approaches.

**Conclusions**

In reaction mixtures with trigonelline, we could detect succinate semialdehyde and succinate along with three new metabolites, which were produced on a preparative scale, purified and structurally characterized by 1D- and 2-D NMR. Unexpectedly, the first intermediate exhibited a rare 5-hydroxy-butyrolactone structure in equilibrium with an open form, which resulted from a successive reduction-oxidation and cleavage of the pyridine ring followed by a recyclization. This reaction scheme remains unprecedented for a natural pyridine compound and may have valuable spin-off effects in the field of bioremediation since pesticide derivatives or pyridine itself may also be catabolized by this way.

In addition, we showed that trigonelline metabolism is connected with the metabolism of compatible solutes in the cell, extending its physiological function in bacteria beyond nutrition.

## **FEMS-1321**

### **Secondary metabolites, metabolomics**

#### **MICROBIAL VOLATILES – SMALL COMPOUNDS WITH POWERFUL ACTIVITIES**

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#### **Background**

Soil microorganisms produce a range of secondary metabolites like antibiotics, toxins, biosurfactants, siderophores. Beside these metabolites soil microorganisms are capable of emitting another class of secondary metabolites so called volatile organic compounds (VOCs). Volatiles are low molecular mass compounds (100-300 Da) with high vapor pressures, low boiling points and lipophilic character. These properties facilitate evaporation and diffusion through both water- and gas-filled pores in soil and rhizosphere environments. As compare to other microbial secondary metabolites volatiles are relatively less studied.

#### **Objectives**

The major aim of our study was to obtain more insight in the role of volatiles in microbial interactions.

#### **Methods**

In order to obtain insight in the importance of volatiles in the inter-specific interactions between soil microorganisms we performed several experiments in glass Petri dishes plates, which were designed as such that growth of different microorganisms occurred in physically separated areas within a common atmosphere. In this way we studied the role of volatiles in fungal-bacterial and bacterial-bacterial interactions. The volatiles were analysed by using thermal desorption GSMS.

#### **Conclusions**

The obtained results revealed that volatiles play important role in the interaction between soil microorganisms. Here we will report on bacterial volatiles with (1) antimicrobial activity and (2) on volatiles acting as infochemicals affecting the behavior, growth and gene expression in responding bacteria. Furthermore we will report on the effect of fungal volatiles on bacteria and on the importance of interspecific interactions for the production of volatile.



**FEMS-1061**

**Secondary metabolites, metabolomics**

## **ANALYSIS OF THE DRAFT WHOLE GENOME SEQUENCE OF THE HUMIDIMYCIN PRODUCER STREPTOMYCES HUMIDUS F-100.629**

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### **Background**

Characterization of new secondary metabolite biosynthetic clusters is gaining increased attention because of their potential to produce new molecules with medical and biotechnological application. Most biosynthetic pathways in actinobacteria are cryptic or silenced in standard laboratory conditions, requiring alternative approaches to identify and these new clusters.

Humidimycin (MDN-0010) is a peptide structurally related to the antiviral siamycins that potentiates the antifungal activity of caspofungin against *Aspergillus fumigatus* and *Candida albicans* (1). Humidimycin is produced by the strain *Streptomyces humidus* F-100.629.

### **Objectives**

This study has been focused on a gene mining of *S. humidus* F-100.629 to identify the humidimycin biosynthetic pathway and other potential cryptic biosynthetic gene clusters.

### **Methods**

Whole genome sequence was obtained with Illumina. Assembly and annotation were performed using standard bioinformatics tools.

### **Conclusions**

The draft genome size of *S. humidus* F-100.629 was estimated in 8.4 Mb. The biosynthetic cluster of humidimycin was identified and 50 putative secondary metabolite clusters were predicted using the antiSMASH application (2), including PKS-I, PKS-II, NRPS, terpenes, siderophores and bacteriocin, suggesting the genomic potential of the strain as producer of diverse natural products.

1-Valiante et al. (2015) Hitting the caspofungin salvage pathway of human-pathogenic fungi with the novel peptide humidimycin (MDN-0010)(submitted).

2-Blin, K. *et al.* (2013) antiSMASH 2.0--a versatile platform for genome mining of secondary metabolite producers. *Nucleic Acids Res.* 41:W204–212.



**FEMS-2971**

**Secondary metabolites, metabolomics**

## **MICROEVOLUTION OF THE BIOACTIVE PEPTIDE ANABAENOPEPTIN IN CYANOBACTERIA**

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### **Background**

Bloom-forming cyanobacteria such as the genus *Planktothrix* sp. form a large number of bioactive peptides, e.g. the family of the Anabaenopeptins (AP) through nonribosomal peptide synthesis (NRPS).

### **Objectives**

In total 140 *Planktothrix* strains, isolated from various climatic zones, were phylogenetically assigned into three major lineages that differed in ecosystem type colonization. We analyzed the distribution and recombinations of the AP synthesis gene cluster (*apn*).

### **Methods**

The presence or absence of the *apn* gene cluster was determined by polymerase chain reaction (PCR) and sequencing of PCR products.

### **Conclusions**

We found that the majority of strains of lineage 1, occurring in shallow lakes, carried specific *apn* remnants in parallel to the full gene cluster. The recombinations occurred within adenylation domains resulting in rare structural variants as well as a fusion of part of the ABC transporter domain from another NRPS gene cluster. In contrast, strains of lineage 2, isolated from deep lakes, did not contain *apn* remnants but always contained the full gene cluster. Strains of lineage 3, isolated from tropical regions, did not carry any sequence related to *apn*. Overall evolutionary diversification of *apn* genes was congruent with ecological diversification. Because of the occurrence of *apn* remnants and recombination events within the *apn* in lineage 1 we hypothesize that its ancestor lost the *apn* genes but some of the genotypes regained it through horizontal transfer.

**FEMS-0924**

**Secondary metabolites, metabolomics**

**A NEW GENE CLUSTER FOR MYCOSPORINE-LIKE AMINO ACID BIOSYNTHESIS IN CYANOBACTERIUM: HETEROLOGOUS EXPRESSION, PURIFICATION, AND CHARACTERIZATION**

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**Background**

Mycosporine-like Amino Acids (MAAs) are an important class of secondary metabolites known for their protection against ultra violet (UV) radiation and other cellular functions, such as scavenging of free oxygen radicals, regulation of osmotic balance, and resistance to thermal and salt stress and desiccation. Cyanobacteria are microorganisms that colonize diverse ecological niches exposed to the UV light and produce MAAs. Because of its efficient sunscreen role, the MAA shinorine from cyanobacteria is utilized commercially in sunscreen creams.

**Objectives**

The biosynthesis of MAA was recently solved and showed that the structural diversity is directly encoded by the gene cluster. Bioinformatic analysis of the genome of the soil strain *Cylindrospermum stagnale* revealed a new gene cluster homolog to the MAAs biosynthetic gene cluster. Analysis of this gene cluster presents five genes instead of three/four common biosynthetic genes in other cyanobacteria.

**Methods**

The gene cluster was successfully cloned and heterologously expressed in *E. coli*. The MAA was extracted from recombinant *E.coli* cultures and identified through HPLC and a UV absorption maxima of 310 nm. Structural characterization is underway, combining the data from mass spectrometry and NMR.

**Conclusions**

The characterization of MAA gene cluster from *Cylindrospermum stagnale* shows a new gene organization suggesting that it codes for a novel biosynthesis pathway. The new MAA, potentially produced by this terrestrial strain, might play a role for combating the terrestrial environmental stress(s).



**FEMS-2949**

**Secondary metabolites, metabolomics**

## **ENHANCED PHYCOCYANIN PRODUCTION, USING NITROGEN-FIXING NOSTOC SP. WITH CHROMATIC ACCLIMATION**

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### **Background**

Phycocyanin (PC) is the major pigment of the phycobiliproteins in cyanobacteria. The commercial applications of PC could be divided into two sections; the first is an ingredient for nutrient elements, natural dyes for food and cosmetics, and the second is a potential remedy in oxidative stress-induced diseases.

### **Objectives**

The aim of this study was to increase the PC content in the isolated cyanobacterium (*Nostoc* sp.) with nitrogen-free medium and chromatic acclimation.

### **Methods**

*Nostoc* sp. was cultured for 10 days in 1-L cylinder flasks containing 800 ml nitrogen-free BG11 medium. For chromatic acclimation, two types of light sources were compared, i.e. plant-fluorescent lamp (only blue and red wavelengths are provided) and white fluorescent lamp.

### **Conclusions**

Under fluorescent lamp condition, the maximum biomass production was 2.24 g DCW/L (dry cell weight g/L). High biomass production could be achieved by nitrogen fixation of *Nostoc* sp., even without nitrogen supply. The maximum biomass concentration was higher at 2.65 g DCW/L under plant lamp condition. It means that blue and red lights in plant lamp are more critical in promoting cell growth. Cell color also changed from brown to green through chromatic acclimation by plant lamp. Higher PC content (15%) in cells was obtained and maintained throughout the cultivation. By changing light source from white to plant lamp, biomass production and PC content have been increased. Cost-effective PC production was possible,

due to its nitrogen fixation ability. More simplified PC extraction process was also devised for more efficient PC production.

**FEMS-2255**

**Secondary metabolites, metabolomics**

## **ENDOPHYTIC MICROBIOTA OF THE AMAZON: ANTIBIOTIC ACTION OF METABOLITES IN PATHOGENS PRESENT IN WOUND**

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### **Background**

The endophytic microorganisms (EM) of the Amazon are potential producers of secondary metabolites with biological activity that can be used on infected wounds.

### **Objectives**

This research investigated the antibiotic action of ME from the Amazon front of pathogenic microorganisms present in the in wound healing process.

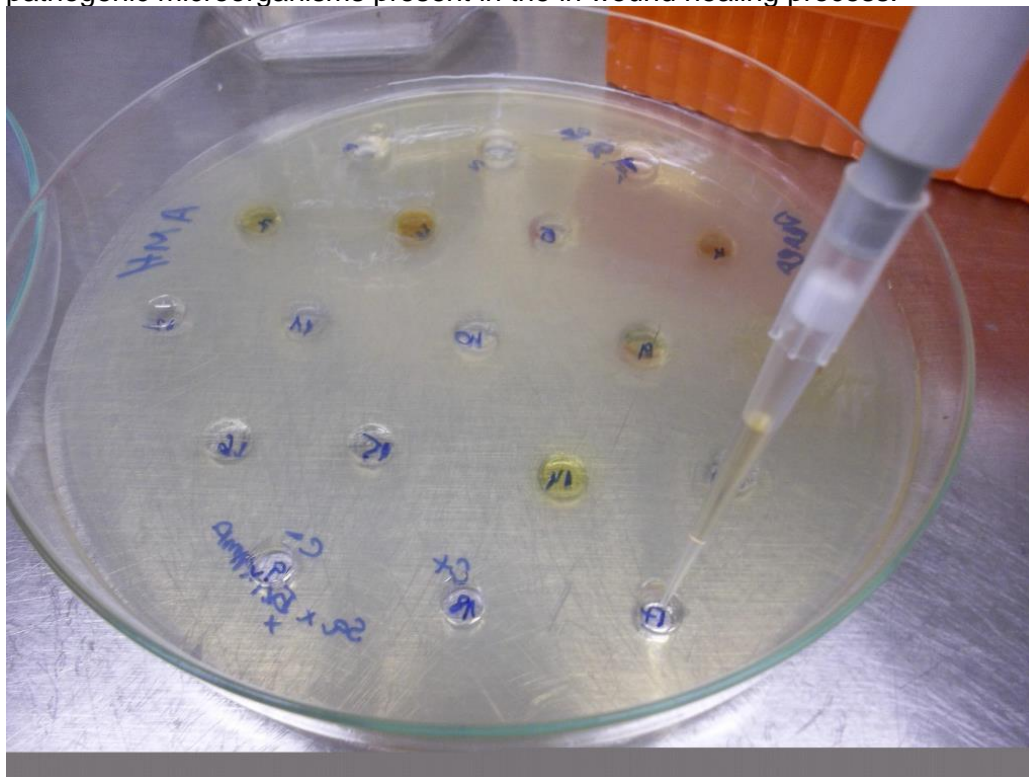


FIG 01.

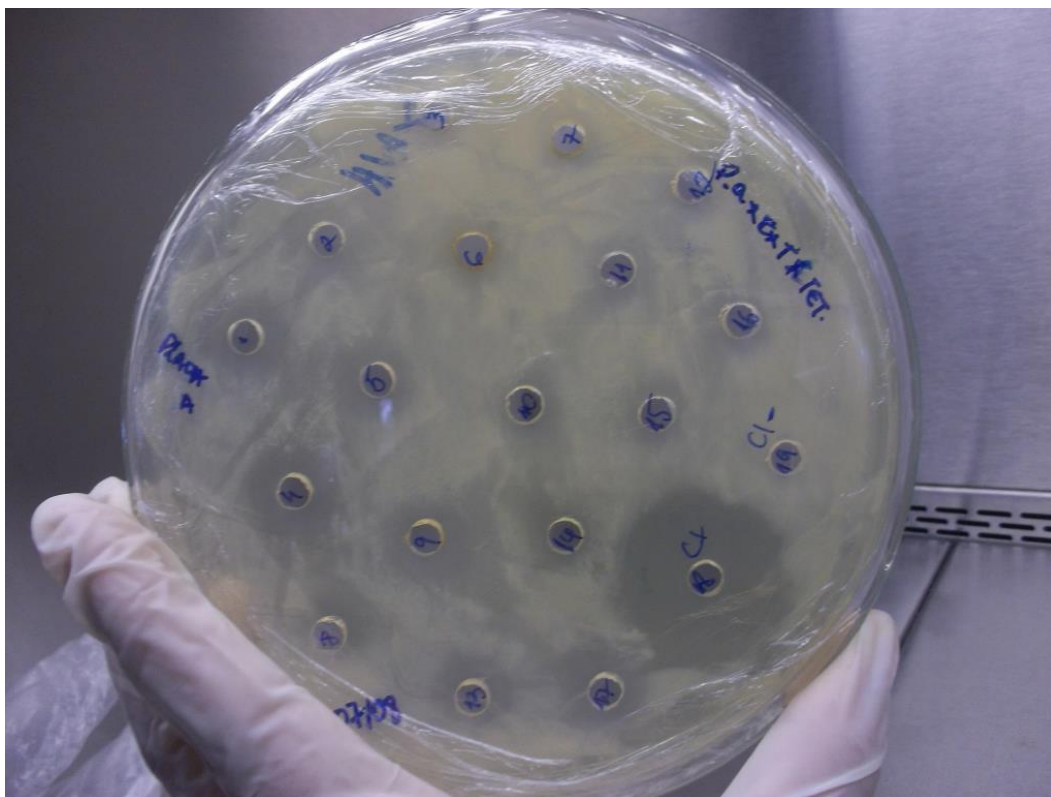


FIG 02.

### Methods

The antibiotic activity of 171 secondary metabolites of endophytic microorganisms (MSME) were analyzed against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Candida albicans* by diffusion in agar. In the test were used 50 $\mu$ L of each MSME extract; ampicillin, tetracycline, ciprofloxacin and fluconazole, all in 2% as positive control according to the test microorganism and DMSO as a negative control. The readings of the zones inhibition were carried out in 24 and 48 hours. The mean, standard deviation, ANOVA and Tukey's test ( $p < 0.05$ ) were performed for analysis.

### Conclusions

The largest zone of inhibition with significant differences for *C. albicans* were MSME DfGa2 1.2 G4 ( $2.88 \pm 0,71\text{cm}$ ) and DfGa2 1.2 G5 ( $2.72 \pm 0,69\text{cm}$ ), for *S. aureus* were MSME DfGa2 1.2 G4 ( $2,52 \pm 0,83\text{cm}$ ) and DfGa2 1.2 G5 ( $2,32 \pm 0,45\text{cm}$ ), for *P. aeruginosa* were MSME DfGa2 1.2 G5 ( $2,24 \pm 0,30\text{cm}$ ). For *E. coli* there was no significant difference between the means of the zones of inhibition. The authors concluded that the samples DfGa 2 1.2 G4 and DfGa 2 1.2 G5 had better antibiotic action for the tested pathogens, suggesting a formulation of a gel and, subsequently, its evaluation *in vivo* of the activity of healing wounds in rats.

**FEMS-0743**

**Secondary metabolites, metabolomics**

**ANTIFUNGAL ACTIVITY OF METABOLITES FROM STREPTOMYCES  
DIASTATOCHROMOGENES (NO.1628) AND ITS ACTIVE PRINCIPLE**

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**Background**

The fermentation broth by *Streptomyces diastatochromogenes* (No.1628), isolated from a soil sample collected in Mountain Tianmu, Hangzhou, China, had strong antifungal activity against *Alternaria solani*, *Botrytis cinerea*, *Fusarium oxysporum* and *Rhizoctonia solani*.

**Objectives**

The aim of this research is to isolation and identification of active compounds from *S. diastatochromogenes* 1628.

**Methods**

A bioactivity-guided approach was employed to isolate and determine the chemical identity of bioactive constituents with antifungal activity from *S. diastatochromogenes* 1628.

**Conclusions**

By the mycelium growth rate assay, it was showed that the n-butanol extract dosedependently inhibited growth of above tested phytopathogenic fungi with a potency at the concentration of 175 mg/L that was equal to that of commonly used antifungal agents at the concentrations tested. Furthermore, n-butanol extract of *S. diastatochromogenes* (No.1628) could effectively suppress and control cucumber rhizoctonia rot caused by *R. solani*. Through bioassay-guided fractionation, the n-butanol extract from *S. diastatochromogenes* (No.1628) afforded a new tetraene macrolide, (7E, 12Z,13E,15E,17E,19E) -21 -((4-amino-3,5-dihydroxy -6-methyltetrahydro -2H -pyran-2-yl)oxy) -12-ethylidene- 1,5,6,25 -tetrahydroxy -11 -methyl-9-oxo-10,27-dioxabi-cyclo[21.3.1]heptacos-7,13,15,17,19-pentaene-24-carboxylic acid (1), together with tetrin B (2), tetramycin A (3), toyocamycin(4) and anisomycin (5). The structures of compounds were established on the basis of spectroscopic analyses. This new compound strongly inhibited hyphal growth of *R. solani* and *B. cinerea* with IC<sub>50</sub> of 0.20 and 1.53 µg/mL, respectively. Our study demonstrated that *S. diastatochromogenes* (No.1628) is a promising source of natural bioactive and novel metabolites and has wide application prospect in biocontrol field.



**FEMS-2681**

**Secondary metabolites, metabolomics**

**PANCREATIC LIPASE INHIBITORY ACTIVITY OF ENDOPHYTIC FUNGUS ASSOCIATED WITH AEGLE MARMELOS: A BIORESOURCE FOR NOVEL ANTI-OBESITY DRUG**

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**Background**

Obesity is a disease resulting from improper balance between energy intake and expenditure and is increasingly becoming a major cause of preventable mortality. Pancreatic lipase (PL) is considered as one of the safest target for diet-induced anti-obesity drug development.

**Objectives**

In the process of exploration of new PL inhibitors, we have screened culture filtrates of 200 endophytic fungi from different medicinal plants using qualitative and quantitative assays. The organism was identified and partial purification of the bioactive moiety was carried out using analytical techniques.

**Methods**

**Rhodamine olive oil plate assay** and PNPL (p-nitrophenyl laurate) as substrate was used for quantitative assessment of PL activity inhibition.

**Conclusions**

The qualitative assays indicated potential PL inhibition in 27 isolates. Further, only organic extract of these culture filtrates exhibited PL inhibition in range between 30-100%. #6 AMLWLS exhibited complete inhibition of PL with an IC<sub>50</sub> of 2.12 µg/ml which was comparable to the Orlistat exhibiting an IC<sub>50</sub> value of 2.73 µg/ml. Purification of the extract by column chromatography led to isolation of the pure compound which was identified as a tetra peptide molecule on the basis of C<sup>13</sup> NMR, HR/MS and biochemical analysis. Further molecular phylogenetic tools and morphological studies were used to identify the isolate #6 AMLWLS as *Fusarium incarnatum* species.

Hence endophytic fungal isolate #6 AMLWLS stands out as a potential candidate for anti-obesity therapy

**FEMS-3086**

**Secondary metabolites, metabolomics**

**SCREENING CYTOTOXICITY AND LEISHMANICIDE ASSESSMENT  
ENDOPHYTIC FUNGI FROM HYPTIS SUAVEOLENS (L) POIT AND COMBRETUM  
LANCEOLATUM POHL**

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**Background**

Bioprospecting using endophytic fungi is a promising source for the detection of novel compounds with biological activities of interest in health care, especially for diseases such as leishmaniasis (neglected tropical diseases) and cancer.

**Objectives**

The objective was to evaluate the cytotoxicity and the leishmanicide activity of endophytic fungi extracts isolated from *H. suaveolens* (L) Poit and *C. lanceolatum* Pohl against tumor cell lines MCF-7 and MDA-MB-231e *Leishmania amazonensis*.

**Methods**

We have evaluated 25 fungal culture extracts obtained by maceration of the mycelium in ethyl acetate (EtOAc). The fungi have isolated from *H. suaveolens* and *C. lanceolatum*. The extracts were tested against tumor cell lines MCF-7 and MDA-MB-231 and promastigotes forms of *L. amazonensis*. The test used was a colorimetric method with tetrazolium salt 3- [4,5-dimethylthiazol-2-yl] -2,5-diphenyltetrazolium bromide (MTT). The concentration of the extracts ranged from 100 to 12.50 µg/mL.

**Conclusions**

Extracts of 5 fungal isolates (*Taifanglania curticaenata*-F27; *Marasmius* sp.-21C, *Fusarium oxysporum*-33C; *Macrophomina phaseolina*-46C and *Trichoderma spirale*-66C) inhibited the proliferation of promastigotes at a low IC(50) of between 25.61 and 52.60 µg/ml. Six extracts (*M. phaseolina*- F1/46C; *T. curticaenata*-F27; *Cladosporium flabelliforme*- F29; *Neosartorya pseudofischeri*-F36, and *Corynespora cassiicola*-72C) were cytotoxic to both cell lines at a low IC(50) of between 23 and 48.72 µg/ml. Our results indicate that the medicinal plants living in wetlands Brazilian shelter an interesting bioactive fungal community that is able to produce leishmanicidal and antitumoral molecules. These molecules may be used to develop new leishmanicidal and anticancer drugs.

Acknowledgements: CNPq and FAPEMAT

**FEMS-1956**

**Secondary metabolites, metabolomics**

**VERIFICATION OF PEPTAIBOL PRODUCTION ABILITY OF A TRICHODERMA STRAIN SELECTED BY A BACTERIUM-BASED SCREENING METHOD**

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**Background**

Filamentous fungi are producers of a large number of valuable secondary metabolites due to their promising biological effects. Identification of these compounds and characterization of their biological activities are the important goals of research working at the interface of chemical and biological sciences.

**Objectives**

We aimed to verify the peptaibol production of a *Trichoderma* strain selected from the Szeged Microbiological Collection by a *Micrococcus luteus*-based agar plate screening method.

**Methods**

Mass spectrometric analysis of putative peptaibols produced by a *T. pleuroticola* strain have been carried out via on-line reversed-phase high performance liquid chromatography (HPLC) coupled to electrospray ionization ion trap mass spectrometry (ESI-IT-MS) after the solid phase clean-up of culture extracts. Initially, the separation and the mass spectrometric parameters were optimized using alamethicin standard peptaibol mixture and the unknown components produced by the selected isolate were investigated and determined.

**Conclusions**

Our mass spectrometric measurements confirmed the results of the biotest selection method used for the detection of peptaibols. Some of the produced peptaibols showed the characteristic mass spectral components of trichorzianins, including TA IIIb, TB IVb, TAP-14a and TA VII. However, besides the known molecules, seven new trichorzianin-related compounds were also detected; these contained some

amino acid changes in the core trichorzianin sequence. Comparing the amount of the different components, compounds with a molecular mass of 1909 amu were present in the highest amount. This research was supported by OTKA grant K-105972 from the Hungarian Scientific Research Fund and a bilateral grant from “Stiftung Aktion Österreich-Ungarn”.

**FEMS-1968**

**Secondary metabolites, metabolomics**

## **PURIFICATION AND STRUCTURAL ELUCIDATION STRATEGY OF PEPTAIBOLS PRODUCED BY TRICHODERMA STRAINS**

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### **Background**

An outstanding group of secondary metabolites are peptaibols, produced mainly by fungal species from the genus *Trichoderma*. These compounds show a wide spectrum of biological activities including antibacterial, antifungal and antiviral effects.

### **Objectives**

We aimed to develop a simple and easy-to-follow peptaibol characterization strategy including purification and structural elucidation steps, which can help to analyze the peptaibol-producing abilities of various isolates within the genus.

### **Methods**

Our characterization strategy includes the mass spectrometric analysis of the culture extracts of possible peptaibol-producing *Trichoderma* strains, the semi-preparative purification of the selected compounds, identification of amino acids in their sequences, and chiral analysis of the identified building blocks. These tasks require the use of microbiological methods for strain identification and for different cultivations and the application of separation techniques such as solid phase extraction, normal and reversed-phase HPLC and mass spectrometry.

### **Conclusions**

During the study, the different methodological steps were optimized and their application served the detailed characterization of some *Trichoderma* strains from the point-of-view of their peptaibol profiles. The examined isolates are belonging to the species *T. aggressivum*, *T. atroviride*, *T. longibrachiatum*, *T. orientale*, *T. pleuroticola*, *T. pleurotum* and *T. virens*. The peptaibol compositions showed high level of diversity and their amounts presented in a wide range. A number of already described compounds have been identified from species other than the original producer and novel peptaibols were also defined. This research was supported by OTKA grant K-105972 from the Hungarian Scientific Research Fund and a bilateral grant from "Stiftung Aktion Österreich-Ungarn".



**FEMS-2008**

**Secondary metabolites, metabolomics**

## **PURIFICATION AND IDENTIFICATION OF FIVE OPHIOBOLIN ANALOGUES FROM *BIPOLARIS ORYZAE***

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### **Background**

Ophiobolins are a remarkable family of secondary metabolites with various biological activities such as calmodulin antagonism, cytotoxic, antimicrobial and nematocid effects.

### **Objectives**

Our aim was to develop a rapid and efficient method for purification of different bioactive sesterterpene-type fungal secondary metabolites belonging to the ophiobolin family.

### **Methods**

Based on our preliminary results, a *Bipolaris oryzae* strain was selected and cultivated in PDB. The ferment broth was extracted with ethyl-acetate and concentrated. The purification of the crude extract was carried out using subsequent semi-preparative liquid chromatographic techniques. Initially, normal phase column chromatography was carried out using the mixture of ethyl-acetate and n-hexane, and the fractions containing large amount of potential ophiobolin compounds were further purified on a semi-preparative reverse phase HPLC system with the mobile phases of acetonitrile and water. After each purification step, the collected fractions were analyzed on an analytical HPLC system to determine their purity. The purified compounds were identified using mass spectrometric and NMR experiments.

### **Conclusions**

The selected *B. oryzae* strain produced several ophiobolin analogues. The purified compounds synthesised in the highest amount proved to be ophiobolin A; its identity was confirmed with HPLC-MS and NMR experiments. Besides ophiobolin A, four other ophiobolin analogues were also purified including 3-anhydro-ophiobolin A,

ophiobolin I, 6-epi-ophiobolin A and 3-anhydro-6-epi-ophiobolin A with the purities over the 95%. This research was supported by grant TÁMOP-4.1.1.C-12/1/KONV-2012-0012. Csaba Vágvolgyi thanks the visiting professor program, Deanship of Scientific Research, King Saud University, Riyadh.



**FEMS-1604**

**Secondary metabolites, metabolomics**

**RECOMBINANT PRODUCTION OF BIOSURFACTANTS WITH ENGINEERED  
PSEUDOMONAS PUTIDA**

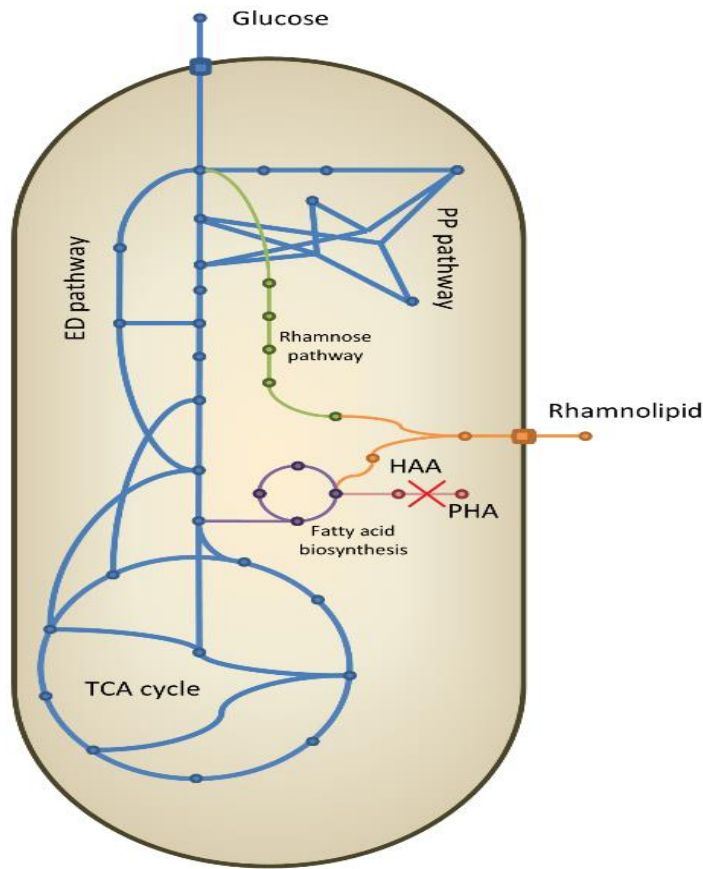
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**Background**

Rhamnolipids are potent biosurfactants with high potential for industrial applications. They are currently produced with the opportunistic pathogen *Pseudomonas aeruginosa* during growth on hydrophobic substrates such as plant oils. The heterologous production of rhamnolipids enables the use of a non-pathogenic host and cheaper substrates such as glucose. Glucose also has the advantage of simpler purification, e.g., via coupled foam fractionation and adsorption [1].

**Objectives**



To engineer a non-pathogenic rhamnolipid producer converting glucose or other renewable resources efficiently into the target product.

## Methods

Introduction of the biosynthesis pathway for rhamnolipids from *P. aeruginosa* in the non-pathogenic *P. putida*. Metabolic engineering of secondary metabolite producers implicitly relies on high flux through central carbon metabolism. Our strategy relies on the ability of *P. putida* to reroute metabolic resources into a peripheral pathway when a high demand exists, e.g., initiated by high transcriptional activity.

## Conclusions

We previously presented a recombinant *P. putida* able to produce 0.2 g/L rhamnolipids [2]. For optimization, several techniques were used. A synthetic promoter library was developed using an approach based on degenerated primers [3]. This technique yields an array of transcriptional activity, potentially allowing the identification of optimal enzyme activity for high flux towards rhamnolipid synthesis. The second step was eliminating a pathway competing for a common precursor. The best producing strain was able to reach a titer of 3 g/L with a yield of 40% and a very high specific rhamnolipid synthesis rate, which is possible because the flux through the precursor providing pathways is significantly increased in the engineered strain.



**FEMS-1413**

**Secondary metabolites, metabolomics**

## **FUNCTIONAL CHARACTERIZATION OF A GENE INVOLVED IN THE BIOSYNTHESIS OF MYCOPHENOLIC ACID IN THE FUNGUS *PENICILLIUM ROQUEFORTI***

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### **Background**

*Penicillium roqueforti* is a filamentous fungus used in the ripening of blue-veined cheeses. Previous studies have shown that this organism produces several secondary metabolites, including mycophenolic acid, a meroterpenoid with immunosuppressive activity of great pharmaceutical interest. However, the genes involved in the synthesis of this metabolite in *P. roqueforti* have not been described or characterized so far. In other organisms such as *P. brevicompactum*, it has been described that the mycophenolic acid biosynthetic cluster consists of seven genes, among them *mpaDE*, which encodes for a natural fusion enzyme consisting of a cytochrome P450 and a hydrolase.

### **Objectives**

To evaluate the role of the *mpaDE* gene in the biosynthesis of mycophenolic acid in *P. roqueforti*.

### **Methods**

The genome of *P. roqueforti* was analyzed using bioinformatic tools and an ORF that corresponds to the hypothetical orthologue of *mpaDE* was identified. The expression of this gene was down regulated using RNAi-silencing technology. For this purpose, *P. roqueforti* was transformed with an appropriate genetic construct, and several transformants strains showing dramatic reductions in the levels of *mpaDE* transcript were selected. Finally, extracts from these transformants were analyzed by HPLC.

### **Conclusions**

*P. roqueforti* wild-type showed high levels of mycophenolic acid. On the contrary, the transformants showed barely detectable levels of this metabolite. These results indicate that *mpaDE* is involved in the biosynthesis of mycophenolic acid in *P. roqueforti*.

This work was supported by FONDECYT 1120833 y DICYT-USACH.



**FEMS-1248**

**Signalling**

**A UNIQUE GLUCOSE SENSOR GCR1 OF THE METHYLOTROPHIC YEAST HANSENULA POLYMORPHA CAN TRANSPORT MONOSACCHARIDES**

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**Background**

Several proteins involved in glucose sensing, signalling and transport have been described for methylotrophic yeast *Hansenula polymorpha*. Among them Gcr1, a peculiar C-tale-less glucose sensor, required for glucose repression and regulation of hexose transport.

**Objectives**

The aim of this work was to further elucidate the *H. polymorpha* Gcr1 protein function in glucose transport.

**Methods**

Wild type NCYC495 (WT) and mutant strains of *H. polymorpha*, classical methods of molecular genetics and microbiology were used.

**Conclusions**

The closest homologue for glucose sensor Gcr1 of *H. polymorpha* is the functional high-affinity monosaccharide transporter involved in H<sup>+</sup>-dependent glucose symport – MstA from *Aspergillus niger*. Regulated *HpGcr1* production in hexose transporter-less *Saccharomyces cerevisiae* mutant did not restore growth of this strain on different glucose concentrations. Similarly to Gcr1, functional high-affinity glucose transporters of *Trichoderma harzianum* Gtt1 and *Aspergillus nidulans* HxtA did not complement growth deficiency of *S. cerevisiae* *hxt null* on different carbohydrate substrates. Grown on methanol-containing medium WT-derivative strains with constitutive expression of *GCR1* and *HXT1* encoding low-affinity hexose transporter were more sensitive to exogenous glucose antimetabolite 2-deoxy-D-glucose (2-DOG) (concentration 0.3 mM) compared to the WT strain. The toxic effect of 2-DOG was more acute on medium with lower pH. Therefore, Gcr1 can possibly transport glucose toxic analogue into cells, thus increasing its harmful effects.

*H. polymorpha* protein Gcr1 that most probably results from a horizontal transfer from *Aspergillus* fungi, is essential for glucose catabolite repression of genes involved in metabolism of alternative carbon substrates and "very-high affinity" glucose transport.

**FEMS-1893**  
**Signalling**

## **DESIGNING FUNCTIONAL HETERODIMERS TO DECIPHER SIGNAL TRANSDUCTION IN BVGS**

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### **Background**

The virulence of *Bordetella pertussis*, the whooping cough agent, is regulated by the two-component system BvgAS. BvgA is a classical response regulator while BvgS is an unorthodox sensor-kinase harboring receiver and Histidine phosphotransfer domains. BvgS contains three putative perception domains, two periplasmic Venus Flytrap domains linked by a transmembrane segment to a cytoplasmic PAS domain. The kinase of BvgS is active by default and is turned off by negative modulators such as nicotinate or sulfate ions.

### **Objectives**

BvgS functions as a homodimer, and its phosphorylation cascade works in trans. We took advantage of this property to construct a merodiploid strain in which only heterodimers, but not homodimers, are functional to decipher the signal transduction mechanism involved in BvgS.

### **Methods**

We introduced point mutations in the various sensor domains to test their effect in heterodimers. The same substitutions in the context of a wild type phosphorylation cascade affect either the basal kinase activity of BvgS or its ability to respond to modulation, as seen using a *lacZ* reporter gene under the control of a virulence gene promoter.

### **Conclusions**

When substitutions affecting the response to modulation are introduced in the periplasmic VFT domains of BvgS, the WT monomer is able to partly compensate for the defect of the other monomer, yielding intermediate phenotypes. In contrast, the substitutions introduced in the PAS domain appear to be dominant over a wild type PAS domain. This suggests that signal transduction is achieved through different mechanisms in the periplasmic and cytoplasmic moieties of BvgS.

**FEMS-1877**  
**Signalling**

**THE STRUCTURE AND FUNCTION OF ISOLATED N-TERMINAL AMPHIPATHIC  
HELIXES OF MEMBRANE STRESS CONTROLLERS PSPA AND VIPP1**

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**Background**

All cell types maintain the integrity of their membrane. One widely distributed plasma membrane stress response system in bacteria is the phage shock protein (Psp) system. Its central component PspA has a counterpart Vipp1. Both proteins are directly responsible for membrane maintenance under stress conditions while PspA also negatively regulates its own expression via interaction with the AAA+ ATPase PspF.

**Objectives**

The PspA and Vipp1 N-terminal amphipathic helix A (ahA) is implicated in their membrane binding, and ahB in PspA is required for negative regulation. A direct interaction between ahA and the lipid bilayer for effector function and ahB and PspF for regulatory function has yet to be determined.

**Methods**

Purified short peptides derived from the amphipathic helices were used to directly probe membrane binding and regulatory function. Defined membrane vesicle *in vitro* assays and CD spectroscopy characterised the functionalities of the ahs and the downstream effects on membrane stability and transcription regulation.

**Conclusions**

We observed direct membrane-binding of ahA derived peptides and in the case of Vipp1 an accompanying change in secondary structure from random coil to alpha



helical upon membrane association. Binding specificity by varying the membrane anionic lipid content and stored curvature elastic stress linked the functionalities of the peptides with those of full length proteins. ahB of PspA inhibited the ATPase of PspF proving its direct regulatory role. These findings establish synthetic peptides can probe PspA structure-function and target regulation of the Psp response, which could be of interest for controlling pathogens where Psp response is important and conserved.

**FEMS-2756**  
**Signalling**

## **NEW INSIGHTS IN THE ACTIVATION OF THE PSEUDOMONAS AERUGINOSA VIRULENCE REGULATOR SIGMA VREI AND THE PUMA3 CELL-SURFACE SIGNALING SYSTEM**

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### **Background**

Cell-surface signaling (CSS) is a regulatory mechanism used by gram-negative bacteria to modify gene expression in response to environmental signals. CSS regulation is extensively present in the human opportunistic pathogen *Pseudomonas aeruginosa*. Most CSS systems of this bacterium regulate iron uptake. However, the PUMA3 CSS system regulates virulence. *P. aeruginosa* PUMA3 is constituted by the VreA receptor, the VreR anti-sigma factor and the  $\sigma^{\text{Vrel}}$  extracytoplasmic function (ECF) sigma factor. VreR binds to and keeps inactive  $\sigma^{\text{Vrel}}$  in absence of the PUMA3 inducing signal. The role of VreA is to sense the presence of the signal and to transduce it to the VreR anti-sigma factor allowing the release and activation of  $\sigma^{\text{Vrel}}$  and the expression of the PUMA3 regulated genes.

### **Objectives**

The PUMA3 inducing signal is still unknown. However it has been recently shown that expression of the *vreAIR* operon is induced in low phosphate (Pi). In this condition, some degree of  $\sigma^{\text{Vrel}}$  activity is also observed despite the fact that the VreR anti-sigma factor is also produced. In this work, we aimed at analyse the role of VreA and VreR in both the expression and the activation of  $\sigma^{\text{Vrel}}$  in low Pi.

### **Methods**

*P. aeruginosa* *vreR* and *vreA* deletion mutants has been constructed by allelic exchange. Gene expression was monitored using *lacZ* transcriptional fusions.  $\sigma^{\text{Vrel}}$  stability has been assayed by Western-blot.

### **Conclusions**

Full activation and stability of  $\sigma^{\text{Vrel}}$  in low Pi requires the removal of VreR and the presence of an additional inducing signal that targets this process.



**FEMS-1599**  
**Signalling**

## **IMPACT OF POTENTIAL IN VIVO DIVK DIMERIZATION ON CAULOBACTER CRESCENTUS CELL CYCLE**

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### **Background**

Response regulators are crucial actors of two-component systems and have often been shown to dimerize. We focus on DivK, an essential single domain response regulator of the aquatic  $\alpha$ -proteobacterium *C. crescentus*. DivK is part of a phosphorelay controlling developmental events and belongs to the same protein subfamily as CheY and Spo0F, two response regulators known to dimerize. A previous study has highlighted DivK dimerization *in vitro* (Guillet *et al.*, The Journal of Biological Chemistry, 2002) but the relevance of this dimerization *in vivo* is still a mystery.

### **Objectives**

The goal of our research is (i) to assess whether DivK is able to dimerize *in vivo* and (ii) to determine whether this dimerization mediates DivK subcellular localization and/or the interaction with potential partners. We are also interested in investigating the importance of DivK phosphorylation in its dimerization.

### **Methods**

Under native conditions, DivK appears as a doublet which is not visible under reducing conditions. Interestingly, the relative intensity of the DivK bands varies when the PleC phosphatase or the DivJ kinase, both acting on DivK, are knocked-out. In addition, the doublet is upshifted in the DivK<sub>D90G</sub> mutant, whose subcellular localization is affected. The nature of this doublet is currently under investigation. We are also trying to identify DivK partners by MS-MS analysis of DivK co-immunoprecipitates obtained from various genetic backgrounds.

### **Conclusions**

Preliminary results suggest that DivK is indeed able to dimerize *in vivo* and to bind different partners. This dimerization could be triggered by phosphorylation.



**FEMS-2171**  
**Small regulatory RNAs**

**USE OF ANTISENSE RNA TO MODULATE HSP GENE EXPRESSION IN  
OENOCOCCUS OENI**

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**Background**

*Oenococcus oeni* is a wine-associated lactic acid bacterium responsible for wine malolactic fermentation. Wine exhibits harsh and challenging *conditions*: low pH, low temperature, nutrient-poor and presence of ethanol leading to *O. oeni* stress response. Understanding of the mechanisms involved in stress tolerance is essential to improve *O. oeni* development in wine.

Stress adaptation of *O. oeni* has been studied and many *hsp* genes, induced in oenological conditions, have been identified. The *hsp18* gene, encoded the small Hsp Lo18, have been particularly characterized. In addition to its chaperon activity, Lo18 could have a lipochaperon-function and maintains membrane integrity in stress conditions.

**Objectives**

Up to now, characterization of *O. oeni* genes was restricted due to lack of genetic tools for gene replacement. Accordingly, we focused our work on gene inactivation by using antisense RNA approach to modulate *hsp* gene expression in *O. oeni*. With the goal of understanding the function of *O. oeni hsp* genes *in vivo*, we have developed an efficient expression vector to produce antisense RNA targeting *hsp18* mRNA and reduce sense RNA transcript.

**Methods**

After transformation into *O. oeni*, transformants were exposed to multiple stresses inducing *hsp18* gene expression: heat shock, acid shock and presence of ethanol.

**Conclusions**

We highlighted that *in vivo* antisense inhibition of *hsp18* expression strongly affects the survival of *O. oeni* in stress conditions. This study presents a new efficient genetic tool for *O. oeni* and demonstrates for the first time the use of antisense technology for modulating gene expression in *O. oeni*.



**FEMS-1555**

**Small regulatory RNAs**

## **DIFFERENTIAL EXPRESSION OF SMALL RNAs IN RESPONSE TO CHEMICAL STRESS**

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### **Background**

Bacterial sRNAs are often expressed in response to changing environmental conditions and function to modulate gene expression. There are numerous documented connections between sRNAs and stress, where sRNAs regulate important processes in response to metabolite, envelope, oxidative, iron deficiency, anaerobic and pH stress.

### **Objectives**

Although chemical stress is routinely encountered in microbial processing applications due to the toxicity of substrate, intermediate or product compounds, the cellular response and the involvement of sRNAs in this process is poorly understood. We have used an RNA sequencing approach to map the *E. coli* sRNome during chemical stress and high cell density fermentations with the aim of gaining insights into the chemical stress response and identifying sRNAs with roles in stress tolerance that have potential applications in the design and optimization of future production strains.

### **Methods**

RNA sequencing libraries were prepared from RNA isolated from *E. coli* MG1655 cells subjected to chemical stress with twelve compounds including organic acids, amino acids, and organic solvent-like compounds. The *E. coli* MG1655 strain was also grown under high cell density fermentation conditions, where cells were harvested in exponential batch, glucose-limited exponential fed-batch, transition and stationary growth phases.

### **Conclusions**

Over 250 novel intergenic sRNAs have been identified with this approach, adding to the roughly 200 previously reported sRNAs in *E. coli*. A significant fraction of the sRNAs exhibit specific expression patterns during high cell density fermentation and



a group of them are differentially expressed in the presence of multiple chemicals, suggesting they may play regulatory roles during these stress conditions.

**FEMS-1568**

**Small regulatory RNAs**

**HFQ BINDING PROFILES OF SRNAS AND THEIR TARGET MRNAS**

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**Background**

Bacterial sRNAs are 50-400 nucleotide-long RNA molecules, most of which exert their regulation by base-pairing with their target mRNAs. In many bacteria, including *Escherichia coli*, the interaction between an sRNA and its targets is mediated by the Hfq protein, resembling the situation in eukaryotic cells where the interaction of a microRNA and its targets involves Argonaute. While it is widely accepted that miRNAs guide Argonaute proteins to their specific targets through sequence complementarity, the dynamics of sRNA-Hfq-target interaction, to the best of our knowledge, has not been yet fully determined.

**Objectives**

As a first step towards this question, we study the relationship between the levels of Hfq-bound sRNA and its target mRNAs. More specifically, we aim to measure the change in Hfq-bound target mRNA levels following a change in the expression level of their sRNA regulator.

**Methods**

To this end we applied RNA-seq to measure the levels of total RNA and RNA co-IPed with Hfq from three *E. coli* strains (MG1655 Hfq-FLAG, MG1655 hfq::kan and MG1655 Hfq-FLAG rnc14::Tn10) with and without overexpression of the sRNA MicA.

**Conclusions**

Our results indicate that most MicA target mRNAs, as well as MicA itself, were enriched in Hfq when MicA was overexpressed. In contrast, most target mRNAs of other sRNAs were depleted. In conclusion, our initial results indicate that there is a relationship between sRNA and target mRNA levels on Hfq, offering a strategy to identify putative novel targets.

**FEMS-1909**

**Small regulatory RNAs**

**THE TRANSCRIPTOME LANDSCAPE OF LACTOCOCCUS LACTIS MG1363:  
SMALL REGULATORY RNAs UNCOVERED**

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**Background**

RNA sequencing has recently revolutionized transcriptome analyses. As a result, novel non-coding RNAs have been discovered in several organisms and subsequent studies have revealed an increasing number of new regulatory mechanisms at the level of RNA. The lactic acid bacterium *Lactococcus lactis* is of high importance for biotechnological applications, specifically in the dairy industry. The stress response systems are very well studied for this bacterium. In addition, global regulators of carbon and nitrogen metabolism have also been studied in detail. However, the presence and roles of non-coding RNAs with regulatory functions has not been assessed until now.

**Objectives**

We aimed to identify novel RNA elements, such as small regulatory RNAs, antisense RNAs and sORFs. In addition, expression of these novel RNAs under various stress conditions was assessed, and for one novel sRNA, the function was studied in depth.

**Methods**

By using differential RNA sequencing (dRNA-seq) data, a transcriptome landscape of *L. lactis* was constructed that was used for manual and automated mining using TSSer. Northern hybridization was used to confirm 12 putative regulatory RNAs.

**Conclusions**

This study used both manual and automated approaches to identify hundreds of novel RNA species. Experimental validation was performed for a selection of these potential RNA regulators. The role of one non-coding RNA located in the 3'UTR of *argR*, the regulator of e.g. the *arc*-operon in *L. lactis*, is currently being examined. This small RNA, called ArgX, appears to stabilize the *arc*-operon. Results of this sRNA ArgX and its role in regulating *arc*, will be presented.



## FEMS-1755

### Small regulatory RNAs

#### A GROUP B STREPTOCOCCUS SMALL RNA PRESERVES CELL INTEGRITY AND CONFERS RESISTANCE TO A CATIONIC ANTIBACTERIAL PEPTIDE

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### Background

Gram-positive bacteria reduces the effectiveness of cationic antimicrobial peptides (CAMPs), such as colistin, and preserves cell integrity by the addition of D-alanine to lipoteichoic acids (LTAs). Thus far, no small RNA (sRNA) has been described to be involved in the regulation of the genes involved in the D-alanylation of LTAs in the human neonatal pathogen Group B *Streptococcus* (GBS).

### Objectives

Here, we present the first study describing the functional characterization of a GBS sRNA. We highlight its pleiotropic role in maintaining cell integrity and colistin resistance.

### Methods

The GBS sRNA was identified by RNAseq and its expression was confirmed by northern blot analyses. The biological role of the sRNA was investigated by analyzing a deletion mutant ( $\Delta srna$ ). The interaction region between the sRNA and its mRNA target was predicted *in silico* and confirmed by *in vivo* mutagenesis.

## Conclusions

The northern blot analyses showed that the sRNA is mainly expressed at the onset of the stationary phase until late stationary phase. Microscopic observations showed that the  $\Delta srna$  strain forms significantly shorter chains, and is more sensitive to colistin (MIC = 16  $\mu\text{g/mL}$ ) when compared to the wild-type (MIC > 256  $\mu\text{g/mL}$ ). Interestingly, the *in silico* predicted mRNA target is downregulated in the mutant strain. Mutations in the sRNA sequence likely involved in RNA-RNA pairing are sufficient to provoke similar chains alterations than the  $\Delta srna$  strain. All together, these results suggest that the sRNA is required for colistin resistance and cell integrity due to a direct regulation of the D-alanylation pathway.

**FEMS-3070**

**Veterinary microbiology**

**DEVELOPMENT OF OAPV3 VIRAL-LIKE PARTICLES FOR INVESTIGATION OF SHEEP HUMORAL IMMUNE RESPONSE TO PAPILLOMAVIRUS INFECTION**

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**Background**

Papillomaviruses are a diverse group of small, non-enveloped, double stranded DNA viruses that cause proliferations of the stratified squamous epithelium of the skin and mucosa in a wide variety of vertebrate hosts. Papillomaviruses play an important role in human cancer development, and have been isolated from a number of animal malignancies. Recently we identified a novel ovine papillomavirus, OaPV3, associated with squamous cell carcinoma. Like other papillomaviruses, OaPV3 is not able to productively infect cells in culture. The lack of *in vitro* factories for viral production hampers the investigation of pathogenetic mechanisms and interaction with host immune response.

**Objectives**

Main goal of this study is the production of non-infectious OaPV3 viral-like particles (VLPs) for evaluating humoral immune response in naturally infected sheep.

**Methods**

Non-infectious VLPs were obtained by expressing the L1 major capsid protein in insect cells using recombinant baculoviruses. Two sheep were immunized with OaPV3 VLPs. Sera were collected after each of the 3 immunisations. VLPs were used in western blotting and ELISA to test reactivity of 90 sera collected from sheep sampled in flocks naturally infected by OaPV3.

**Conclusions**

Data indicate a specific immune response of immunised sheep. In naturally infected flocks, only sheep with clinical lesions showed detectable antibodies. OaPV3, like human cutaneous papillomavirus seems to escape humoral response by playing “hide and seek” with host immune system.





**FEMS-3071**

**Veterinary microbiology**

**GENOME CHARACTERIZATION AND CELLULAR TROPISM OF OAPV4, A NOVEL OVINE PAPILLOMAVIRUS ISOLATED FROM SHEEP FIBROPAPILLOMAS**

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**Background**

Papillomaviruses play an important role in human cancer development, and have been isolated from benign and malignant proliferative skin lesions. However, the association of papillomaviruses with cutaneous lesions has been poorly investigated in sheep. To date, three ovine papillomaviruses types have been classified. OaPV1, OaPV2 belong to *Deltapapillomaviruses* genus and were found in cutaneous fibropapillomas of Australian Merinos. OaPV3 belongs to a new genus, *Dyokappa*, and has been recovered from cutaneous squamous cell carcinomas of Sarda breed sheep.

**Objectives**

In this study we investigated unknown ovine papillomaviruses in cutaneous fibropapillomas of Sarda breed sheep.

**Methods**

Rolling circle amplification was performed on total DNA extracted from skin lesions biopsies, and resulted in the presence of a novel ovine Papillomavirus (OaPV4). The entire OaPV4 genome was cloned and sequenced. IHC with L1 antibodies and ISH with E6 dig-probe were carried out in order to establish viral cellular tropism.

**Conclusions**

A novel papillomavirus, OaPV4, was identified. Viral L1 shares 82.5% nucleotide homology with the closest relative (OaPV1). Maximum likelihood phylogenetic tree placed OaPV4 in a monophyletic clade including all *Deltapapillomaviruses* so far described.

OaPV4 infects both epithelial cells and fibroblasts and has a role only in benign lesion development. This is the first report of a *Deltapapillomavirus* 3 species in European Sarda breed, and this supports the existence of an OaPV1/OaPV4 common ancestor infecting sheep before radiation and evolution of modern sheep breeds.



**FEMS-3075**

**Veterinary microbiology**

## **EQUUS ASINUS PAPILLOMAVIRUS (EAPV1) PROVIDES NEW INSIGHTS INTO EQUINE PAPILLOMAVIRUS DIVERSITY**

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### **Background**

The Papillomaviridae family consists of a large and diverse group of viruses characterised by a double strand, covalently linked circular genome typically ranging from 7 to 8 kb in size, and causing proliferative lesions in animals and human.

### **Objectives**

Aim of this study was to investigate papillomavirus diversity in Equidae and to establish the presence of PV in species related to horse. We detected a novel papillomavirus (EaPV1) from healthy skin and from sun associated cutaneous lesions of an Asinara (Sardinia, Italy) white donkey reared in captivity in a wildlife recovery centre. EaPV1 genome is 7467 bp long, and shows some characteristic elements of horse papillomaviruses, including a small untranslated region between the early and late regions. A typical E6 ORF is missing. EaPV1 DNA was detected in low copies in normal skin of white and grey donkeys of the Asinara Island, and does not transform rodent fibroblasts in standard transformation assays. Pairwise nucleotide alignments and phylogenetic analyses based on concatenated E1-E2-L1 amino acid sequences revealed the highest similarity with the Equine papillomavirus type 1.

### **Methods**

The entire genome of EaPV1 was cloned, sequenced, and characterised. Methods included: DNA extraction, traditional and real-time PCR, RCA, cloning, sequencing and phylogenetic analyses.

### **Conclusions**

The discovery of EaPV1, the prototype of a novel genus and the first papillomavirus isolated in donkeys, confirms a broad diversity in Equidae papillomaviruses. Taken together, data suggest that EaPV1 is a non-malignant papillomavirus adapted to healthy skin of donkeys.



**FEMS-1399**

**Veterinary microbiology**

**BACTERIOSTATIC SYNERGISTIC EFFECT OF RECOMBINANT HUMAN LACTOFERRIN, SILVER AND ZINC NANOPARTICLES ON PATHOGENIC ENTERIC BACTERIA ISOLATED FROM DIARRHEAL CALVES**

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**Background**

The spread of multidrug-resistant Enterobacteriaceae is complicating the treatment of diarrhea in neonatal calves. Growing resistance of microorganisms to antibiotics requires to find novel effective antibacterial drugs.

**Objectives**

To evaluate the bacteriostatic effect of human lactoferrin (hLf), silver nanoparticles (AgNPs), zinc nanoparticles (ZnNPs) and their combinations on pathogenic enteric bacteria isolated from diarrheal calves.

**Methods**

Recombinant hLf was isolated from milk of transgenic goats generated by microinjecting sequence encoding hLf cDNA to the pronuclear. AgNPs and ZnNPs were prepared by chemical methods. The radii of particles were between 20 and 25 nm. *E.coli*, *Pr. mirabilis* and *Kl. pneumoniae* used in these experiments were isolated from diarrheal calves. Isolated bacteria were resistant to ciprofloxacin and erythromycin. To evaluate the bacteriostatic effect of hLf, nanoparticles and their combinations the broth microdilution minimal inhibitory concentration (MIC) testing was applied.

**Conclusions**

The results suggested that the MIC value of AgNPs against *E.coli*, *Pr. mirabilis* and *Kl. pneumoniae* was about 2-4 µg/ml; ZnNPs activity was lower (about 8-16 µg/ml). The synergistic antimicrobial effect between hLf and NPs was also evident. The MICs of hLf decreased when used together with NPs. So the MIC of hLf against all of the tested bacterial strains was reduced from 4-8 mg/ml when used alone to 0,5-2 mg/ml when used in conjunction with 1 µg/ml of AgNPs. The synergistic activity of hLf and ZnNPs combination was less pronounced. The best synergistic combination was AgNPs and hLf that allows to recommend it for the treatment of diarrhea in neonatal calves.

**FEMS-2137**

**Veterinary microbiology**

**EFFECTS OF COLD STRESS ON BACTERIAL ISOLATION AND IMMUNE CELLS IN CAMPYLOBACTER JEJUNI INFECTED SPF CHICKENS.**

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**Background**

Stress activate the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system resulting in a series of neural and endocrine adaptations. In industrial production the cold stress are one of the most important problem in the first week of poultry production. Many microorganisms are present in the livestock and poultry products are considered main source of *Campylobacter jejuni* to humans.

**Objectives**

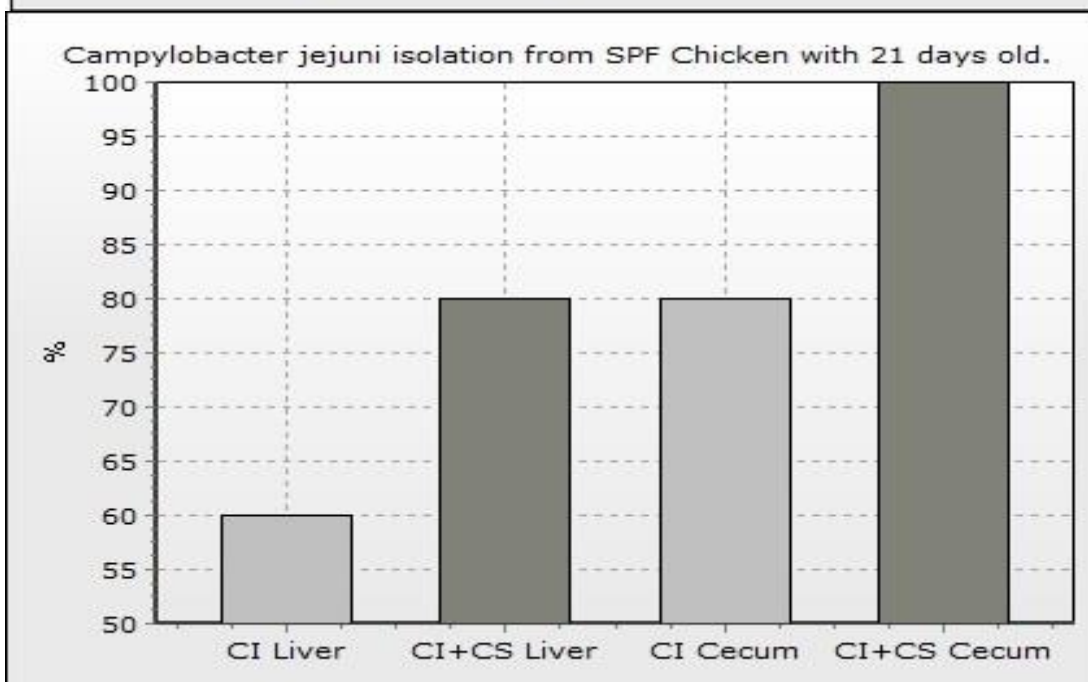
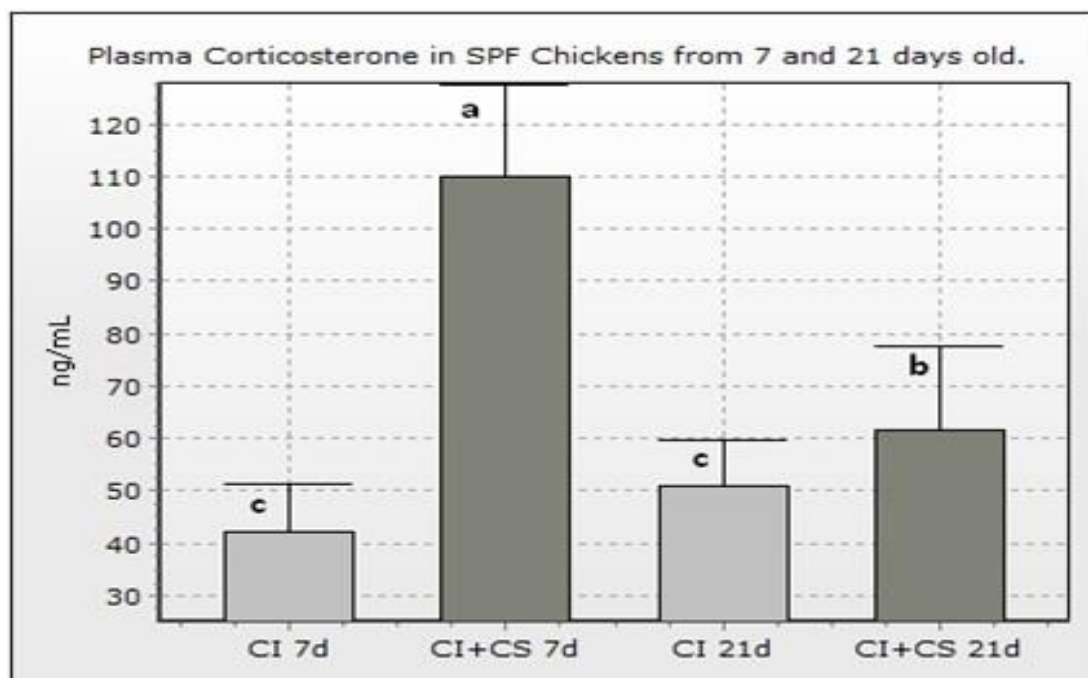
Our aims were challenge chickens with cold stress and *Campylobacter jejuni* (ATCC33291) infection to assess the role of stress in the interaction host-pathogen

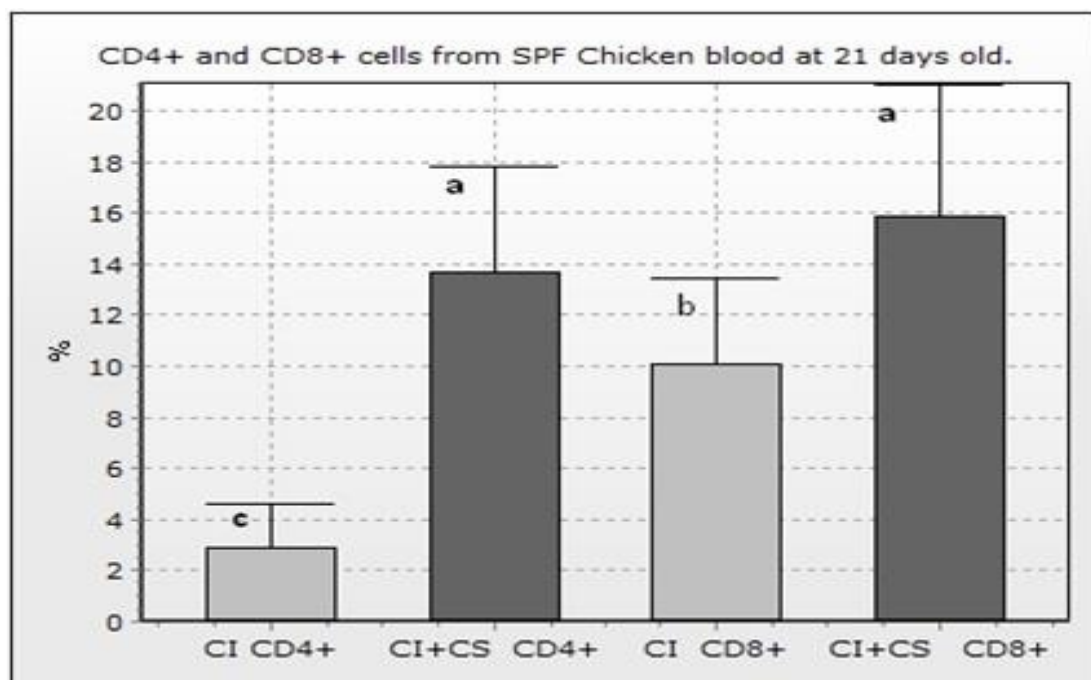
**Methods**

Thirty-two Leghorn SPF birds were housed in isolators divided into three groups: C (negative control not shown), CI (*Campylobacter jejuni* infected -  $10^4$  ufc) kept for 21 days in thermal comfort and the group CI+CS (*Campylobacter jejuni* infected -  $10^4$  ufc + cold stress) was subjected to cold stress for 6 h per day during 7 days at a 19°C. At 7 and 21 days old, bacterial isolation from liver and cecum, blood flow cytometry to CD45, CD3, CD4 and CD8 antibodies, plasma corticosterone (CS) (Fig.1) and blood glucose (BG) levels were performed in the birds.

**Conclusions**

We observed high BG and CS ( $p<0,005$ ) during entire experiment in CI+CS group. Also, the cold stress increased bacterial isolation at 21days in the liver and cecum (Fig. 2). At 21days of age, we observed a increase of CD3+ ( $p<0,05$ ) CD4+ and CD8+ peripheral cells in CI+CS group (Fig.3). We may conclude that high CS and BG in birds subjected to cold stress affect negatively the bacterial clearance and immune cells distribution.







**FEMS-3080**

**Veterinary microbiology**

**MYCOPLASMA AGALACTIAE ESCAPES SHEEP NEUTROPHIL  
EXTRACELLULAR TRAPS (NETS)**

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**Background**

Mycoplasmas are the smallest self-replicating bacteria that evolved a parasitic lifestyle through regressive evolution. The ability of mycoplasmas to modulate immune responses allows the establishment of persistent infection resulting in chronic inflammatory diseases. Mycoplasmas evolved different strategies to escape host immune system. Despite mechanisms of humoral response evasion are known, resistance to innate response is still insufficiently investigated.

To date, studies of interaction between mycoplasmas and granulocytic neutrophils are still lacking. NETs are a mixture of antimicrobial peptides and proteins "caught" in a backbone of DNA filaments that trap bacteria, which are cleared by macrophage through phagocytosis. It has been demonstrated that *Staphylococcus aureus* (*S. aureus*) breaks down NETs through its SNase and induces macrophages apoptosis. Recently, we demonstrated the *in vivo* expression of MAG\_5040, the SNase homologue of *M. agalactiae*. We speculate that MAG\_5040 might also be an important virulence factor of mycoplasmas.

**Objectives**

Main goal of this study is to investigate the interaction of *M. agalactiae* with NETs and to verify if MAG\_5040, similarly to its *S. aureus* homologue, allows *M. agalactiae* to avoid NETs -mediated neutrophils extracellular killing.

**Methods**

Sheep neutrophils, either untreated or activated with PMA and/or MAG\_5040, were incubated with *M. agalactiae*. After 3 hours, extracellular DNA was stained with SYTOX Orange and images were detected with a confocal microscope. Differences in fluorescence intensity were also calculated.

**Conclusions**

Data suggest that *M. agalactiae* is able to induce NETs formation and their digestion *ex vivo*, suggesting a novel mechanism for escaping sheep innate immune response.

**FEMS-2212**

**Veterinary microbiology**

**PERSISTENT CONTAMINATION OF SMALL AND MEDIUM SIZED POULTRY FARM ENVIRONMENTS BY CLOSTRIDIUM PERFRINGENS IN THE UNITED KINGDOM**

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**Background**

*Clostridium perfringens* is a cause for increasing concern due to its involvement in severe infections in both humans and animals, especially poultry. Although *C. perfringens* is part of the natural microbiota of the intestinal tract in poultry, it is an opportunistic bacterium which can be pathogenic resulting in the possibly severe disease - necrotic enteritis.

**Objectives**

The aim of the present study was to determine the occurrence of *C. perfringens* on one single sampling occasion from poultry enterprises widely distributed across the United Kingdom. The samples were toxin genotyped by PCR and analysed by PFGE and antimicrobial testing

**Methods**

172 samples were collected from 50 poultry establishments described as 'free range' operations. None of the enterprises either prior or during the study reported necrotic enteritis in their poultry. Samples for bacterial isolation were grown on tryptose sulphite cycloserine (TSC) agar and quantified; typical colonies of *C. perfringens* putatively identified and confirmed biochemically. The samples were subjected to toxin production identification, ribosome typing, PFGE and antibiotic susceptibility testing.

**Conclusions**

The presence of *cpa*, *cpb*, *etx*, *iA*, *cpb2*, *cpe*, and *NetB* toxin genes were determined by PCR. 89.7% *C. perfringens* isolates were classified as type A and the remaining 10.3% type C; no other toxin genes were identified. Quantitative estimates of numbers of isolates was higher than expected (from 10<sup>4</sup> to 10<sup>9</sup> CFU/g) from poultry

farm sources, regardless of the cleaning protocol used to sanitise the housing environment. Attempts to correlate specific clusters of *C. perfringens* with specific locations will be presented.

**FEMS-1543**

**Veterinary microbiology**

## **MSSA AND MRSA CO-COLONIZATION DYNAMICS AND CLONAL DIVERSITY IN PIGS**

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### **Background**

Methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA) are colonizers of skin and mucosa. Studies about MSSA and MRSA in human patients showed a competition of these two microorganisms for colonization space in the anterior nares. Moreover, in humans one clone can be found rather than differing types of MSSA and MRSA. At present, it is unknown whether this is also true for animals.

### **Objectives**

The aim of this study was to investigate the colonization dynamics of both MSSA and MRSA in pigs over a longer time period and investigate their clonal diversity. The results might be of interest for the development of control strategies. This is important as in farm animals, and particularly in pigs common MRSA are found that are transmissible to humans and the environment.

### **Methods**

Eighteen pigs were sampled three times every ten weeks with a nasal swab. Additionally, environmental samples were taken. All samples were investigated for MSSA and MRSA, respectively. Spa-typing was done with up to five MRSA and MSSA isolates, respectively, found per sample and time point. Of almost 400 MSSA and MRSA isolated, 62 isolates were further investigated by microarray.

### **Conclusions**

The results do not support the hypothesis of a competitive colonization of MSSA and MRSA in the anterior nares of pigs. Rather we found a changing status. Hence, highly identical clones of MSSA and MRSA are present in the anterior nares of pigs and their environment. CC398 and CC9 associated spa-types were the predominating clonal lineages found among MRSA and MSSA isolates, respectively.

**FEMS-1351**

**Veterinary microbiology**

## **VAGINAL BACTERIOLOGY RESULTS OF THE EWES INDUCED WITH DIFFERENT PARTURITION INDUCTION METHODS**

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### **Background**

Induction of parturition can result in some infective postpartum vaginal complications in ewes due to their side effects on parturition. Therefore, it is important to reveal whether induction methods responsible to the vaginal bacterial changing in ewes.

### **Objectives**

This study aims to reveal vaginal bacteriology and antibiogram results of the ewes induced with different parturition induction methods.

### **Methods**

Twenty-four curly-fleeced ewes (n=24) at 3rd pregnancy period on 138th day were divided as group I (n=6): control; group II (n=6): dexamethasone (16 mg, im); group III (n=6): aglepristone (5mg/kg, im) and group IV (n=6): dexamethasone (8mg, im) + aglepristone (2.5 mg/kg, im). For bacteriological examination, the vaginal swaps were taken from ewes at 138th pregnancy day and then they were induced as below. Parturitions had not any clinical complications. Vaginal swaps were repeated on days of postpartum 15 and 30. Bacterial identifications revealed that *Escherichia coli* was the dominant pathogen bacteria both pre-induction and postpartum days in all groups. In postpartum 15<sup>th</sup> day, *Acinetobacter* spp. was the other pathogens for only in group III. And in postpartum 30<sup>th</sup> day, *Acinetobacter baumannii* was the other pathogen in group III. Antibiotic susceptibility of these pathogens was performed by Kirby Bauer disc diffusion methods. *Escherichia coli* and *Acinetobacter baumannii* were 100.0% sensitive to enrofloxacin, *Acinetobacter* spp. was 100.0% sensitive to trimethoprim / sulfamethoxazole.

### **Conclusions**

It has been considered that induction of parturition can affect the vaginal flora and can be responsible for the postpartum vaginal complications to be encountered.



FEMS-2576

Veterinary microbiology

## CHARACTERISTICS AND ANTIMICROBIAL SUSCEPTIBILITY OF TRUEPERELLA PYOGENES ISOLATED FROM BOVINE MASTITIS CASES IN CHINA

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### Background

*Trueperella (T) pyogenes* is an opportunistic pathogen causes suppurative diseases in dairy cows. However, the pathogenesis and antibiotics resistance of *T. pyogenes* are still unclear.

### Objectives

*T. pyogenes* isolates morphological characteristics, the presence of genes (*plo*, *cbpA*, *fimA*, *fimC*, *fimE*, *fimG*, *nanH*, *nanP*, *tetW*, *ermX*, *ermB*), biofilm formation, the cytopathological effects in intracellular assay and antimicrobial resistance were investigated.

### Methods

*T. pyogenes* was isolated from 50 out of 275 clinical and subclinical bovine mastitis cases in China. Both pyolysin (*plo*) and collagen-binding protein (*cbpA*) virulence factor genes were detected by conventional PCR in all *T. pyogenes* isolates. The tissue culture plate method was used to assay the capacity of *T. pyogenes* for biofilm formation and showed that 90% of *T. pyogenes* isolates were able to form biofilms with different production amount. Minimum inhibitory concentrations (MICs) of 14 antimicrobial agents were determined and observed high susceptibility to rifampin (96%), while high resistance to trimethoprim–sulfamethoxazole (90%) and bacitracin (98%). Intracellular assay revealed that 4 different *T. pyogenes* isolates had different cytopathological effects on infected bovine mammary gland epithelial cells.

### Conclusions

18% *T. pyogenes* isolates indicates that *T. pyogenes* is important contributors to bovine mastitis. Moreover, the within-host quantitative, spatial, high occurrence of multidrug-resistant, biofilm producing and temporal dynamics of *T. pyogenes* interactions are key factors to better understand how immunity acts on infections with bacteria and how they evade immune surveillance; thus, highlighting the need for prudent use of antimicrobial agents in veterinary medicine (This research was supported by projects No. 2012BAD12B03, No. 313054, No. 20120008110042, No. 2014M561102 and No. GDT20141100043).

**FEMS-2980**

**Veterinary microbiology**

### **BRUCELLOSIS IN GREY SEAL IN BALTIC SEA**

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### **Background**

Finnish Food Safety Authority Evira participates in the HELCOM monitoring program to assess the health status of seals in the Baltic Sea area. Organ samples are collected from hunted seals or, to a lesser extent, from seals found dead. In the year 2013 we detected for the first time brucellosis in one of these samples. There are two marine *Brucella* species, *B. pinnipedialis* affecting pinnipeds (sea lions, walrus and true seals) and *B. ceti* affecting cetaceans (dolphins, porpoises and whales). Brucellosis is a zoonosis.

### **Objectives**

The aim of this study was to examine the distribution and possible infection routes of brucellosis in grey seals in the northern Baltic Sea.

### **Methods**

Livers of grey seals (*Halichoerus grypus*) (n=95) were inspected for parasites and pathological lesions. Samples for bacteriological examination (n=18) were taken from livers with macroscopic inflammatory lesions (n=10) and from normal livers (n=8) for control and cultivated on fastidious anaerobe agar plates. The plates were incubated at 37 °C in microaerophilic atmosphere, and observed for bacterial growth daily for 10 d. The identification was based on Stamp stain, biochemical tests and PCR.

### **Conclusions**

Brucellosis does not seem to be rare in grey seals in the northern Baltic Sea. Of 10 samples with lesions 3 were positive. All normal livers were negative. The bile ducts of positive livers contained abundant numbers of flukes (*Pseudamphistomum truncatum*), which were positive for *Brucella* sp. in PCR. Flukes might be one of the possible vectors of brucellosis in marine environments.





**FEMS-1396**

**Veterinary microbiology**

**IDENTIFICATION AND GENETIC CHARACTERISATION OF OVINE  
PARATUBERCULOSIS IN SLAUGHTERED ANIMALS IN SHIRAZ-IRAN USING  
IS900 PCR**

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<sup>2</sup>Food Microbiology, school of Vet Med, Shiraz, Iran

**Background**

*Mycobacterium avium* subspecies *paratuberculosis* (MAP) is an obligate, Gram positive, acid-fast bacterium which has adapted to the gastrointestinal tract of ruminants and other animals and can cause fatal inflammatory disorder called Johne's disease. The potency of microorganism to infect human and its close association with Crohn's disease is the main public health issue of the infection, worldwide.

**Objectives**

The aim of this study was to find out some information about the frequency of MAP in the slaughtered animals and further characterization of its genome, in Southern Iran.

**Methods**

The study was conducted between October 2013 to August 2014 in which total of 450 specimens from ileocecal lymph node, ileocecal valve and surfaces of 150 slaughtered sheep were collected. The animals were categorized according to the sex and age. IS900 PCR assay was employed to confirm the positive cases.

**Conclusions**

Out of 450 specimens, 12 were found positive using IS900 PCR assay. All the positive samples were from the intestinal tissues and lymph nodes. Statistical analysis of the results, using Fisher's Exact test, confirmed no significant difference between the frequency of infections in the ram and ewe. Furthermore, infection in the lambs was significantly lower than sheep over 1.5 year age. In general, employing the IS900 PCR assay is recommended to monitor the MAP infected carcasses. The sequence analysis of the positive cases showed a 93% identity when it was compared with gene bank database.

**FEMS-3103**

**Veterinary microbiology**

## **EAR INFECTIONS IN DOGS IN ICELAND. THE CAUSATIVE AGENTS AND THEIR SENSITIVITY TO ANTIBIOTICS**

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### **Background**

Ear infections are a common problem in dogs. The infections can be very persistent and difficult to treat. The common treatment is with antibiotics and in chronic infections the dogs go through multiple treatments.

### **Objectives**

The aim of this study was to map the most common causes of ear infections in dogs and test their resistance to antibiotics.

### **Methods**

In the period of 2010 to 2013 the Department of Microbiology received 246 samples from dogs for microbial culture. Of these 93 were ear samples and we managed to isolate pathogenic bacteria and/or fungi from 83 of dogs. In the study period the main cause of ear infections in dogs' ears was the bacterium *Staphylococcus intermedius*. In addition we commonly isolated *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Candida spp*. The antibiotic sensitivity of the isolated pathogens varied considerably and we often saw multiresistant *Proteus mirabilis* and *Candida spp*.

### **Conclusions**

Dogs with ear infections are usually treated with antibiotics as soon as the infection is detected. Our study shows that multi-resistant bacteria are common in dogs with recurrent ear infections. It is very important to detect the pathogen and obtain information about antibiotic sensitivity before starting treatment. This will ensure more effective treatment and possibly prevent chronic ear infections caused by multiresistant bacteria.

**FEMS-1091**

**Veterinary microbiology**

**GENOTYPIC RELATEDNESS OF SALMONELLA ENTERICA SEROVAR ENTERITIDIS ISOLATES FROM CHICKENS AND HUMANS IN KOREA**

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**Background**

*Salmonella enterica* serovar Enteritidis is one of the most common serotypes implicated in *Salmonella* infections in both humans and poultry worldwide. Most *Salmonella* infections in humans are considered to be associated with foods of animal origins contaminated by *Salmonella*. Indeed, the most common sources of *Salmonella* Enteritidis infection are believed to be chickens and chicken products.

**Objectives**

This study was to estimate the genetic relationship between *Salmonella* Enteritidis isolates from chickens and humans in South Korea.

**Methods**

A total of 127 chicken isolates were collected from clinical cases, on-farm feces, and slaughtered chicken meat between 1998 and 2012 and 20 human clinical isolates were obtained from patients who showed clinical signs between 2000 and 2006 in Korea. We compared pulsed-field gel electrophoresis (PFGE) patterns and multilocus variable-number tandem-repeat analysis (MLVA) profiles of the isolates.

**Conclusions**

PFGE analysis with all the isolates revealed 28 patterns and MLVA identified 16 allelic profiles. PFGE and MLVA showed common types shared by most human and chicken isolates although there was relatively poor congruence between the two analyses. Overall findings demonstrate the transmission of the serovar Enteritidis between humans and chickens.

**FEMS-2318**

**Veterinary microbiology**

## **DEVELOPMENT AND PERFORMANCE EVALUATION OF ENZYME LINKED IMMUNOSORBENT ASSAY AND LINEBLOT FOR SEROLOGICAL DIAGNOSIS OF LEISHMANIASIS IN DOGS**

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### **Background**

Canine leishmaniasis is a zoonotic disease by the protozoan parasite *Leishmania* transmitted by the bite of an infected phlebotomine sandfly. *Leishmania infantum* is the most common and important cause of canine leishmaniasis worldwide. Other *Leishmania* spp. reported from dogs include *L. mexicana*, *L. donovani*, and *L. braziliensis*. Leishmaniasis can be categorized by two types of diseases in dogs: a cutaneous reaction and a visceral reaction also known as black fever, the most severe form of *leishmaniasis*.

Infection does not invariably lead to illness. In fact, most infected dogs remain asymptomatic and may never develop clinical manifestations. In endemic regions, the prevalence of disease is often less than 10% and only about 1 in 5 infected dogs are considered likely to develop clinical disease.

Diagnosis of canine leishmaniasis is based on the presence of clinical signs together with positive specific antibody assay.

### **Objectives**

The aim of this work was to develop a serological ELISA assay to detect IgG and IgM antibodies against *Leishmania* in serum or plasma samples derived from all mammals.

### **Methods**

Microtiterplates were coated with antigen preparations of *Leishmania infantum*. The presents of antibodies against *Leishmania* is detected by protein A/G-HRP. A sample collection of about 200 positive samples and 400 negative samples was used for development and evaluation of the assay.

## **Conclusions**

Here we show the performance characteristic of the newly developed assay. Due to the improved antigen design, purification method and test setup a superior assay performance was achieved compared to other test methods.

**FEMS-2305**

**Veterinary microbiology**

## **PREVALENCE OF BOVINE MASTITIS PATHOGENS AND ITS ANTIMICROBIAL RESISTANCE IN CHINA**

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### **Background**

Bovine mastitis was one of the common and frequent disease in cows, caused huge economic losses to dairy industry.

### **Objectives**

The objective of this study was to investigate the distribution of bovine mastitis pathogens and their antibiotic resistance in China.

### **Methods**

A total of 3941 milk samples from clinical mastitis cows (n=963), subclinical mastitis cows (n=2772) and healthy cows (n=206), which were collected from 40 cities in 150 dairy farms in China. Bacteria were isolated and identified. And disc diffusion test (K-B method) was used to determine the antibiotic resistance of the main pathogens, including *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Staphylococcus aureus* and *Escherichia coli*.

### **Conclusions**

The results showed that 24 species of bacteria and fungi include 4337 microorganisms were isolated from 3445 milk samples (87.41%), the other 496 milk samples were detected as negative ones. A total of 2386 bacteria were identified as bovine mastitis related pathogens: *Streptococcus agalactiae* (35.54%), *Streptococcus dysgalactiae* (23.55%), *Staphylococcus aureus* (16.89%), *Escherichia coli* (12.99%), *Streptococcus* (5.28%), *Klebsiella* (1.63%), *Proteus* (1.59%), *Pseudomonas aeruginosa* (1.22%), *Stellate Nocardia* (0.59%), *Suppurative Corynebacterium* (0.38%), *Candida albicans* (0.17%) and *Streptococcus pyogenes* (0.17%). The total pathogens detection rate was 62.12%. Drug susceptibility test showed that the four major pathogens have different resistance to all antibiotics, the resistance rate was 10%~100%. Especially to penicillin G, streptomycin, and cotrimoxazole respectively, the resistance rate was 30%~100%.

**FEMS-1160**

**Veterinary microbiology**

## **O SEROGROUPS OF ENTEROPATHOGENIC (EPEC) AND SHIGATOXIGENIC (STEC) ESCHERICHIA COLI FROM**

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### **Background**

*Escherichia coli* producing the attachment-effacement (AE) lesion (EPEC) and/or Shiga toxins (STEC) cause enteritis and (bloody) diarrhoea in young calves and in humans, and are also present in the intestines of healthy cattle. Besides the O157:H7 serotype, EPEC and STEC can belong to more than sixty O serogroups. Of them, 8 have been most frequently identified worldwide: O5, O26, O103, O111, O118, O121, O145 and O165, with some also causing diarrhoea in young calves.

### **Objectives**

This study aimed at identifying the pathotypes and the O serogroups of STEC and EPEC isolated from <1-month-old diarrhoeic calves in Wallonia, Belgium.

### **Methods**

(i) 233 enterohaemolysin-producing *E. coli* were isolated at ARSIA between November 2008 and February 2014 from diarrhoeic calves after growth on EHLY Medium®. They were tested with a triplex PCR targeting the *stx1*, *stx2* (Shiga toxins) and *eae* (AE lesion) genes.

(ii) triplex PCR-positive *E. coli* were assayed with two pentaplex PCR targeting the specific genes coding for the nine O serogroups listed above and for the O104 serogroup.

### **Conclusions**

(i) 206 isolates tested positive with the triplex PCR. The most frequent pathotypes were *eae+stx1+* (102 isolates), *eae+* (78 isolates) and *eae+stx1+stx2+* (13 isolates).



(ii) the most frequent serogroups of EPEC and STEC were O26 (57 isolates) and O111 (36 isolates). A few additional isolates tested positive for the O103, O5, O145, O121 and O157 serogroups.

(iii) the future is to compare these EPEC and STEC with those isolated from healthy cattle and from humans, to identify host- and age-specific properties.

**FEMS-1156**

**Veterinary microbiology**

**COMPARISON OF BACTERIOLOGICAL AND GENETIC IDENTIFICATION OF MAMMARY PATHOGENS IN MILK SAMPLES FROM COWS WITH CHRONIC MASTITIS IN WALLONIA, BELGIUM**

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**Background**

Mastitis is the inflammation of the mammary gland following infection by most often bacteria. Clinical mastitis is characterized by macroscopic modification of the milk while only high cell counts observed during subclinical mastitis. According to the bacterial species, mastitis can also be subdivided as either contagious (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Mycoplasma bovis*, coagulase-negative staphylococci, *Corynebacterium* sp., etc) or environmental (*Escherichia coli* and coliforms, *Streptococcus uberis*, *Streptococcus dysgalactiae*, *Enterococcus* sp., etc).

**Objectives**

The overall objective of this study is to compare the results of detection of mastitis-associated bacteria by classical bacteriology and by quantitative (q) PCR.

**Methods**

A total of 120 milk samples were collected from mammary gland quarters with high cell counts (> 300.000 Somatic Cell) between January and March 2014 in seven farms in Wallonia. Samples were inoculated onto Columbia 5% sheep blood, McConkey's, Chapman's and modified Edwards' agar plates. Isolated colonies were picked up and stored at -80°C till further use. After new growth, colonies were Gram stained and identified using appropriate API sets.

**Conclusions**

A total of 231 isolates grew on Columbia blood agar. Of them, 133 isolates also grew on Chapman's agar including 63 *S. aureus* and 63 CNS; 70 isolates on Edwards' agar including 14 *S. agalactiae*, 12 *S. uberis*, 6 *S. dysgalactiae* and 10 *Enterococcus* sp.; and 15 isolates on McConkey's agar including 3 *E. coli*, 3 *Klebsiella* sp. and 8 *Pantoea* sp. These results are being compared with the results of the qPCR detection assay.



**FEMS-2413**

**Veterinary microbiology**

**DIVERSITY OF STAPHYLOCOCCUS SPECIES, ANTIMICROBIAL SUSCEPTIBILITY AND PREVALENCE OF ENTEROTOXIN GENES AMONG ISOLATES FROM CLINICAL MASTITIS IN KOSOVO**

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**Background**

Mastitis is one of the most frequent and costly disease in dairy cattle caused by different bacteria.

**Objectives**

The purpose of this study was to determine and compare the prevalence of different staphylococci, their antimicrobial susceptibility and toxin production, obtained from mammary infected cows in Kosovo.

**Methods**

Total 152 cow's samples with clinical mastitis were collected. Cows were of different breeds and different lactation. Eight different commonly used antibiotics were analyzed for antimicrobial activity against staphylococci while SET-RPLA kit for enterotoxin detection.

**Conclusions**

Our results showed that, staphylococci were present in 89 out of 154 cows from which 58 were coagulase negative and 31 coagulase positive. *S. aureus* was most dominant (27/89) followed by *S. epidermidis* (25/89) and *S. chromogenes* (15/89). Interestingly, staphylococci were found more frequent in some dairy cow breeds than others. Most of the strains (76/89) were resistant to two or more different antibiotics. The highest resistance was observed against penicillin and ampicillin (>65%). While the lowest resistance was against erythromycin (<3%). Regarding the enterotoxin production, 40 out of the 89 strains produced at least one of the toxin types with SEA and SEC being the most common. In Kosovo is very common antibiotic misuse because of lack of local laboratories, hence the study suggests that, the uncontrolled use of antibiotics in treatment of different infective diseases might cause resistance against many antimicrobials. Therefore, attention should be paid on selection of antibiotics; timing of treatment, dosage and the uncontrolled and careless use of antibiotics should be more strictly regulated.

**FEMS-1245**

**Veterinary microbiology**

## **MALDI-TOF AS A TOOL IN IDENTIFICATION OF AVIAN MYCOPLASMA SPECIES**

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### **Background**

MALDI-TOF is a key method in identification of microorganisms. Large databases of reference and field strains have been made commercially available.

Identifying *Mycoplasma* species has always been a time-consuming process using serological tests and molecular methods which require a large number of primers.

### **Objectives**

Presently, only 3 avian *Mycoplasma* species are available in commercial databases. To evaluate if MALDI-TOF is a useful tool, first a larger database needed to be constructed. Secondly, strains isolated from infected birds were compared and matched against the database.

### **Methods**

Fourteen reference and 8 field strains, obtained from collections held at our facility and from the University of Liverpool were grown in broth. An ethanol/formic acid extraction method was performed on each strain and then spotted on a MALDI target plate. For each strain, minimum 20 mass spectra of high quality were acquired to create the MSP using MALDI Biotyper 3.1 software.

Forty-nine clinical isolates from poultry and wild birds were matched. Twenty-seven were correctly identified with a matching score of  $\geq 2.0$ , 6 with a score between  $\geq 1.7$ -1.99 and 16 remained unidentified.

### **Conclusions**

MALDI-TOF is a rapid and useful tool in identifying *Mycoplasma* species. Fourteen isolates did not match probably due to missing strains in the database or possible unknown *Mycoplasma* species. More clinical isolates will be tested in the future and all non-identified isolates will be verified with 16S sequencing.

### **Acknowledgments**

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**FEMS-1768**

**Veterinary microbiology**

## **IN VITRO ACTIVITY OF CONVENTIONAL DRUGS AND NATURAL ESSENCES AGAINST PROTOTHECA**

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### **Background**

Bovine mastitis caused by *Prototheca* can assume high significance because of economic losses and the potential risk to public health. Many studies highlighted the high level of antibiotic resistance of *Prototheca*, as well as the unsuccessful therapies.

### **Objectives**

To determine the *in vitro* susceptibility of *Prototheca* isolates to different antibiotics and alternative treatments based on essential oils.

### **Methods**

Sixteen isolates of *Prototheca* were identified by multiplex PCR reaction, typed using RAPD-PCR, and tested by the disk diffusion method for their susceptibility to 28 antibiotics. The efficacy tests to essential oils (thymol, carvacrol and cinnamaldehyde) were performed using emitters releasing compounds in a controlled way in jars, containing the *Prototheca* strains streaked on Sabouraud agar.

### **Conclusions**

All the strains were resistant to Amikacin, Ampicilin, Aztreonam, Cefepime, Ceftazidime, Chloramphenicol, Ciprofloxacin, Erythromycin, Fosfomicin, Imipenem, Levofloxacin, Meropenem, Mupirocin, Nitrofurantoin, Oxacillin, Penicilin G, Piperacillin, Quinupristin/Dalfopristin, Rinfamicin, Streptomycin, Tetracycline, Ticarcillin/clavulanic acid, Tobramycin and Vancomycin. In contrast, Colistin sulphate, Gentamicin, Kanamycin and Netilmicin were effective against all the strains tested.

The growth of *P. zopfii* genotype 1- 2, and *P. blasckea* was completely inhibited by thymol, carvacrol and cinnamaldehyde.

The results of the study revealed 4 out of 28 antibiotics tested efficient against *Prototheca* spp. while all the strains were inhibited by the tested essential oils.

Thus, *in vivo* studies are needed to evaluate if these compounds could rise as alternative treatments for bovine mastitis caused by *Prototheca* spp.



**FEMS-1823**

**Veterinary microbiology**

## **CHARACTERIZATION OF VIRULENCE FACTORS AND IMMUNE-STIMULATING ANTIGENS IN *VIBRIO SALMONICIDA***

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### **Background**

*Vibrio salmonicida* is the cause of cold-water vibriosis in farmed Atlantic salmon. The disease manifests as a hemorrhagic septicemia, with a mortality ranging from 3-90 %. In the 1980s, the disease was a major challenge to the Norwegian aquaculture industry, but it is now being effectively controlled by the use of oil-based vaccines. Nevertheless, in recent years there have been some reports of disease outbreaks. In 2013, 13 Norwegian fish farms were diagnosed with cold-water vibriosis, all localized in the three northernmost counties of Norway.

### **Objectives**

The knowledge on virulence factors of *Vibrio salmonicida* and the subsequent immune response of the fish is limited. As bacteria adapt to their current environment, a bacterium isolated from a diseased fish will not be identical to the same species cultivated *in vitro*. The aim of this study was to explore the phenotype of bacteria present *in vivo*.

### **Methods**

We have grown *Vibrio salmonicida* in semi-permeable implants in live fish and analyzed bacterial protein expression by two-dimensional gel electrophoresis and tandem mass spectrometry. The experiment was approved by the Norwegian Animal Research Authority (approval no. ID6228).

### **Conclusions**

Our findings may contribute to the knowledge on disease progression in cold-water vibriosis, as well as to provide novel targets for more effective vaccines. Also, the

study may provide insights in the course of disease for other bacterial infections in fish.

**FEMS-0968**

**Veterinary microbiology**

## **IDENTIFICATION AND TYPING OF LACTOCOCCUS GARVIEAE BASED ON MALDI TOF MS**

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### **Background**

MALDI-TOF-mass spectrometry (MS) has emerged as a powerful technique for the routine identification of microorganisms in clinical microbiology laboratories allowing an easier and faster diagnosis than conventional phenotypic and molecular methods. We have evaluated the suitability of MALDI-TOF for the rapid identification of *Lactococcus garvieae* the etiological agent of lactococcosis, a septicemic infection affecting different wild and farmed fish species, and recognized also as an opportunistic emerging human pathogen.

### **Objectives**

Assessment of the reliability of MALDI-TOF MS for the identification of *Lactococcus garvieae*

### **Methods**

A total of 35 isolates from different host and environments were obtained from the culture collection of VISAVET were used in the study. The identification results obtained by MALDI TOF were compared with those obtained by biochemical identification (API 32 Strep) and by a species- specific PCR assay (Zlotkin et al. 1998. J Clin Microbiol 36, 983-985). Mass spectra acquisition and analysis was performed on a Bruker UltraFlex platform (Bruker Daltonics) using MALDI Biotyper™ 3.0 software in the automatic mode using a matrix of saturated solution of  $\alpha$ -HCCA. In addition, a subset of isolates was subjected to PFGE.

### **Conclusions**

The majority of peaks were obtained in the range from m/z 2000 to 10000. The proteomic results matched with those of genotypic approach. Our results also showed some differences in MS spectra in *L. garvieae* isolates recovered from different origins/hosts suggesting the possible identification of molecular biomarkers for *L. garvieae*. Our study demonstrates that proteomics identification using MALDI TOF MS could be a reliable approach for identifying and discriminating this microorganism.



**FEMS-0946**

**Veterinary microbiology**

**IDENTIFICATION OF ARCANOBACTERIUM PLURANIMALIUM BY MALDI-TOF MS, BY SEQUENCING 16S RDNA AND GENE PLA AND BY DEVELOPMENT OF A LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (LAMP) ASSAY**

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**Background**

Genus *Arcanobacterium* consists of four species namely *A. pluranimalium*, *A. haemolyticum*, *A. phocae* and *A. hippocoleae*. More recently, *A. canis* and *A. phocisimile* were described as novel species. *A. pluranimalium* was initially recovered from a harbour porpoise and a fallow deer, from a dog with pyoderma, from abortion material, semen, abscesses, viscera, navel illness and peritonitis of sheep and from milk samples of cows with mastitis. More recently, an *A. pluranimalium* strain was recovered following necropsy of a juvenile giraffe. However, the clinical importance of *A. pluranimalium* in animal infection remains unclear.

**Objectives**

Because of this widespread presence of *A. pluranimalium* in clinical samples of various animals there is an urgent need of phenotypic and genotypic tests for a rapid and reliable identification of this bacterial species.

**Methods**

The *A. pluranimalium* strains investigated in the present study were analysed phenotypically, by MALDI-TOF MS analysis and by 16S rDNA sequencing. In addition, the previously sequenced gene *pla* encoding pluranimaliumlysin of *A. pluranimalium* was used for molecular identification of this bacterial species and for the development of oligonucleotide primers for a loop-mediated isothermal amplification (LAMP) assay.

**Conclusions**

Phenotypic methods, MALDI-TOF MS analysis, sequencing 16S rDNA and target gene *pla* and the newly designed *pla* LAMP assay of the present study allowed the identification of all nine investigated *A. pluranimalium* strains of ovine and bovine

origin and from a juvenile giraffe. This phenotypic and genotypic approach might help to elucidate the role this species plays in animal infections.

**FEMS-1123**

**Veterinary microbiology**

## **SEQUENCING AND PHYLOGENETIC ANALYSIS OF GP51 GENE OF BOVINE LEUKEMIA VIRUS ISOLATES IN KOREA IN 2014**

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### **Background**

Enzootic bovine leucosis was first reported by serological method in Korea as early as in 1980s and recent prevalence in dairy cattle was over 50%. So far there has been no report on the bovine leukemia virus (BLV) genotype circulating in Korea since partial *env* gene sequence which belonged to genotype 1 was deposited in 2009.

### **Objectives**

This study was performed to identify the genetic diversity of BLV in Korea.

### **Methods**

Total 185 whole blood samples were collected from dairy cattle in two farms in southeast region in Korea. BLV gp51 genes were amplified for diagnosis as described previously by Fechner et al. Gp51 full gene (903bp) for sequencing was amplified. The gp51 PCR products were purified, sequenced and analyzed using DNASTar program and Mega 6 Program.

### **Conclusions**

Total 185 samples were analyzed with PCR, and total 78 samples were positive for BLV *env* DNA nested PCR (37 samples out of 119 in farm A and 41 out of 66 in farm B). Further PCR reaction to amplify full gp51 gene was performed. 19 out of 37 positive samples from farm A and 31 out of 41 positive samples from farm B were amplified successfully. The genotype of sequences from farm A was divided into two types, genotype 1 and genotype 3. All 31 isolates from farm B were found to belong to genotype 1. This is the first report of BLV genotype 3 circulation in Korea.

**FEMS-1125**

**Veterinary microbiology**

**DEVELOPMENT OF COMPETITIVE ELISA FOR THE DETECTION OF ANTIBODIES AGAINST SEVERE FEVER WITH THROMBOCYTOPENIA SYNDROME VIRUS (SFTSV) IN SUSCEPTIBLE ANIMALS**

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**Background**

The Severe Fever with Thrombocytopenia Syndrome (SFTS) caused by SFTS virus (SFTSV) which belongs to the *phlebovirus* in bunyaviridae family is an emerging tick-borne mediated infectious disease for human, and the fatality rate is 12% in China. SFTSV seems to have wide host range in that SFTSV positive ticks were isolated from farm animals and wild rodents. Therefore, it is important to monitor SFTSV positive animals to prevent circulation or transmission of SFTSV.

**Objectives**

As there is no gold standard method to detect SFTSV specific antibodies from field animals, we are developing competitive enzyme-linked immunosorbent assay (c-ELISA) technique using nucleocapsid protein (NP) of SFTSV.

**Methods**

The recombinant NP of SFTSV was obtained by the expression of cloned genes in *E. coli* and the SFTSV was amplified in Vero cells for 7 days. The purified recombinant protein or formalin-inactivated SFTSV was used to immunize laboratory animals. Mono- and polyclonal antibody were tested for their antigen specificity in immunofluorescence assay (IFA) and western blot.

**Conclusions**

We developed direct c-ELISA using SFTSV specific monoclonal antibody, and generated the SFTSV positive serum from immunized cattle. The c-ELISA result shows 98.08% agreement in IFA result using 418 cattle field serum samples. We also observed the cELISA is applicable to detect SFTSV positive sera from immunized cattle, chicken and goat. This technique could be usefully used as a serological diagnostic method for various species of SFTSV susceptible field animals.



**FEMS-2081**

**Veterinary microbiology**

## **PHYLOGENETIC ANALYSIS AND MOLECULAR CHARACTERIZATION OF MYCOPLASMA BOVIS OUTBREAK STRAINS IN DENMARK**

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### **Background**

*Mycoplasma bovis* is a cattle pathogen causing broad spectrum of diseases including mastitis, pneumonia and arthritis. The infection is very difficult to control due to long-time persistence of the organism in asymptomatic carriers, lack of knowledge on the specific role of environmental factors in the development of clinical disease, poor response to antibiotic treatment and the lack of effective immunotherapy.

### **Objectives**

Here we report whole-genome sequence analysis of 41 *Mycoplasma bovis* isolates collected during recent outbreak of disease in Danish cattle herds. Our objectives were to determine the extent of genomic diversity in the test population, identify dominant clones, reconstruct their phylogenetic ancestry and infer transmission history.

### **Methods**

Genome sequencing was performed on an Ion Torrent PGM sequencer. Genomic libraries were prepared by using Ion Xpress Plus fragment library kit and amplified using Ion PGM Template 200 OT2 kit according to manufacturer's instruction. Raw sequence reads were pre-processed using FastQC, mapped to reference genome (PG45) using Burrows-Wheeler aligner and single nucleotide polymorphisms (SNPs) were identified using REALPHY server.

### **Conclusions**

Unique molecular markers were identified for individual strains. Phylogenetic clustering based on SNPs revealed two distinct clades indicating a recent common ancestry. Substantial variations in both the number and sequence identities of individual variable surface proteins were observed with respect to type strain PG45 that may have contributed to increased virulence. The genetic distances inferred from SNPs and temporal distances (sampling dates) showed a strong

negative correlation suggesting that introduction of strains into the herds took place much before sampling time.

**FEMS-2891**

**Veterinary microbiology**

## **UNDERSTANDING THE AETIOLOGY AND INFECTION RESERVOIRS OF DIGITAL DERMATITIS IN BEEF CATTLE AND SHEEP**

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### **Background**

Bovine digital dermatitis (BDD) and Contagious ovine digital dermatitis (CODD), are infectious foot diseases causing severe lameness in cattle and sheep, respectively. The causative agents of digital dermatitis (DD) in dairy cattle are considered to be *Treponema* bacteria, however little work has been done regarding DD in beef cattle. Similarly, although it is suggested CODD emerged from BDD little has been done to investigate the aetiology of the disease in sheep or the transmission of DD.

### **Objectives**

1. Isolate and characterise beef cattle and sheep DD treponemes from lesions and compare with dairy cattle DD treponemes.
2. Identify reservoirs of infection of DD.

### **Methods**

Beef cattle and sheep DD lesions were subjected to culture and PCR analysis for the commonly detected DD treponemes from dairy cattle lesions.

The gastrointestinal (GI) tract of beef cows and sheep was investigated as an infection reservoir of DD treponemes by analysing GI tract tissues for the presence of treponemes. Farm equipment which comes into contact with lesions was analysed by PCR and culture methods for the presence of treponemes.

### **Conclusions**

From 16S rRNA gene analysis, treponemes isolated from beef cattle and sheep lesions belonged to the groups of treponemes associated with dairy cattle lesions. The close similarity poses concerns for species-species transmission.

Several GI tract tissues were positive for DD treponemes, providing evidence towards the GI tract as an infection reservoir for DD treponemes. Additionally, farm equipment

was also found to harbour DD treponemes after coming into contact with an infected foot indicating a possible transmission route.

**FEMS-2958**

**Veterinary microbiology**

## **PROTECTING CHICKENS AGAINST CAMPYLOBACTER JEJUNI BY CAMELIDAE-DERIVED NANOBODIES**

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### **Background**

*Campylobacter jejuni* is a Gram-negative microaerophilic bacterium, and is the major cause of human gastro-enteritis worldwide. It is a zoonotic pathogen and chickens are generally accepted as the most important source of infections. Since the use of antibiotics in animal feeds is no longer an acceptable option and the prevention is limited to hygiene measures, novel control methods are needed.

### **Objectives**

The purpose of this research is to investigate whether colonization of chickens by *C. jejuni* can be controlled by passive vaccination in a cost effective manner by using nanobodies, the antigen-binding domains of heavy-chain antibodies.

### **Methods**

Anti-*Campylobacter* nanobodies were identified after immunization of an alpaca with heat-killed *C. jejuni* cells. The binding of these nanobodies on different *C. jejuni* strains, isolated from chickens and their environment as well as human clinical isolates, was verified. Nanobodies that are able to bind all of the tested strains were selected for further research. We presumed that these nanobodies recognize conserved epitopes expressed on *C. jejuni*. The ability of one of these nanobodies to agglutinate *Campylobacter* cells was tested. This nanobody was multimerised by linking it to magnetic beads. The results confirm that the nanobody recognizes antigens on living *C. jejuni* cells and that multimers of this nanobody are able to agglutinate the *C. jejuni* cells.

### **Conclusions**

The nanobodies with a broad *Campylobacter* specificity can be interesting for diagnostic and therapeutic applications, such as for the reduction or inhibition of the colonization of chickens by *C. jejuni*.

**FEMS-1733**

**Veterinary microbiology**

## **GENETICS AND BIOFILM ANALYSIS ON STRAINS OF E.COLI ISOLATED FROM ANIMALS WITH DIARRHOEIC PROBLEMS.**

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### **Background**

- *Escherichia coli* (*E. coli*) is a bacterium that commonly lives in the intestines of people and animals. There are four major categories of diarrhoeogenic *E. coli*, namely: enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC). These categories of *E. coli* differ in their epidemiology and pathogenesis and their O: H serotypes and for the presence or absence of several genes responsible for their virulence.

### **Objectives**

- The objective of the study was the analysis of virulent genes and biofilm production in *E.coli* strains isolated from animals with diarrhoeic problems.

### **Methods**

The strains were isolated from stool samples of animals affected by diarrhoea. Genetic analysis were performed by multiplex PCRs. Biofilm assays were performed by safranin method with O.D. readings at 492 nm on 96 wells plate.

### **Conclusions**

The isolates were all coming from symptomatic animals from farms in which repeated episodes of diarrhoea are present. All isolates were negative for the *aggR* gene (Aggregative adherence fimbria), the *pet* gene (Plasmid-encoded toxin), *aap* (dispersin) and other genes. 10 out of 35 were positive for *irp2* (yersinia bactin biosynthesis gene) and 18 out of 35 strains carried the *astA* gene which encodes for EAST1 (Enteric Aggregative heat-Stable Toxin1). This protein is thought to play a role in EAEC pathogenicity even if not all EAEC strains harbour the *astA* gene. The analysis for biofilm formations on the first 22 isolates, showed low capability in biofilm formation. Only 8/22 showed values above 120 O.D., but further analysis will be performed with different stains.





**FEMS-1392**

**Veterinary microbiology**

## **DETERMINATION OF ANTIMICROBIAL RESISTANCE AND VIRULENCE GENES PATTERNS AMONG CAMPYLOBACTER FROM BOILERS IN POLAND**

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### **Background**

*Campylobacter* is the most common zoonotic agent deriving from broiler flocks and broiler carcasses in Poland. The increased resistance of this bacteria to antibiotics is a matter of special concern, representing a significant public health problem. The genetic features responsible for pathogenesis of campylobacteriosis are still under investigation.

### **Objectives**

The aims of the study were to test *Campylobacter* strains for antimicrobial resistance patterns and to screen for the presence of virulence-associated genes involved in different steps of infection.

### **Methods**

During the project, 29 broiler farms located in 15 voivodeships all over Poland were selected for sampling at the slaughterhouse level. In total, 252 isolates from caeca (150 *C. jejuni* and 102 *C. coli*) and 197 strains from the corresponding carcasses (142 *C. jejuni* and 55 *C. coli*) were tested. A microbroth dilution method was used to establish the antimicrobial resistance by the minimum inhibitory concentrations (MICs) to 7 antimicrobials. Additionally, the following genes: *cadF*, *flaA*, *virB11*, *iam*, *flhA*, *cdtA*, *cdtB*, *cdtC*, *ciaB*, *docA* and *wlaN* were detected by PCR.

### **Conclusions**

The most common resistance profile of *C. jejuni* was ciprofloxacin-nalidixic acid and tetracyclines. *C. coli* were mostly resistant to ciprofloxacin-nalidixic acid-tetracycline and streptomycin. The results regarding virulence patterns demonstrated that the majority of *C. jejuni* strains had virulence and cytotoxin gene pattern: *flaA*, *flhA*, *cadF*, *ciaB*, *cdtA*, *cdtB*, *cdtC* and *docA*. Among *C. coli*, the simultaneous presence of the *flaA*, *flhA*, *cadF*, *ciaB*, *cdtA*, *cdtB*, *iam* and *docA* markers was most often found.

**FEMS-0782**

**Veterinary microbiology**

**PRESENCE OF CAMPYLOBACTER JEJUNI IN DIFERENT SPECIES OF ANIMALS, POSSIBILITY OF ITS INFLUENCE AS A RESERVOIR AND/OR VECTOR INFECTION OF BROILERS**

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**Background**

Campylobacteriosis is the most common foodborne gastroenteritis in industrialized countries and the number of cases is growing continuously. This bacterial infection is mainly related to the consumption of poultry meat, when it is contaminated by *Campilobacter jejuni*. Although it is a known fact that *C. jejuni* has a highly prevalent in broiler flocks, the source of infection in herds remains still unclear. Nowadays, it would be interesting to knowledge the role of the different animals in the transmission of the infection

**Objectives**

The objective of this research was to determine the prevalence of bacteria in different types of animal species

**Methods**

Both birds and mammals remaining in direct or indirect contact with infected broilers for *C. jejuni* were analyzed. Individual animals were sampled by rectal swab and all samples were processed similarly, being the positive samples confirmed by PCR. This organism was isolated from all species that had been in direct contact with infected broilers, but it cannot be isolated from the animals not having direct contact with them.

**Conclusions**

According to the results obtained, it is concluded that *C. jejuni* can be transmitted between different animal species, acting as vector and / or reservoirs of this organism in broilers.

**FEMS-1313**

**Viral infections and host**

**DETECTION OF HUMAN PAPILLOMAVIRUS DNA AND P53 CODON 72  
POLYMORPHISM IN PROSTATE CANCER IN TURKISH POPULATION**

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**Background:** The possible relationship between human papillomavirus infection, tumor suppressor gene p53 and prostate cancer (PCa) has been previously investigated, however, the etiological role of HPV infection and p53 codon 72 polymorphism in the PCa pathogenesis remains highly controversial.

**Objectives:** The aim of the study was to assess the possible role of human papillomavirus in the development of prostate cancer and the distribution of the p53 codon 72 polymorphism in Turkish population.

**Methods:** We investigated the presence of HPV in 60 formalin fixed and paraffin embedded prostate cancer tissues, as well as in 36 benign prostatic hyperplasia tissue (BPH) samples by using real-time PCR and pyrosequencing. Furthermore, the same group of patients were also screened for the presence of the Arg72Pro polymorphism of the p53 gene, a p53 polymorphism related to HPV.

**Conclusions:** No HPV DNA was detected while using real-time PCR in any of the 36 BPH samples. One of the sixty (1.67%) PCa samples was found to be positive for HPV DNA by using a real-time PCR. After using pyrosequencing analysis, the only HPV type detected in the PCa sample was HPV-57. Compared to the control group, Arg /Pro genotype was observed more frequently in prostate cancer patients ( $p=0.044$ ), and also Pro allele frequency is higher in prostate cancer patients ( $p=0.021$ ). Our findings do not support the involvement of HPV in the etiology of prostate carcinogenesis, and the proline allele could be a risk factor for the development of prostate cancer in the Turkish population.

**FEMS-1360**

**Viral infections and host**

## **FACTORS AFFECTING MARINE MICROBIAL HOST-VIRUS INTERACTIONS**

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### **Background**

Viruses are the numerically dominant life forms on Earth. They represent a huge reservoir of genetic diversity, infecting organisms from small to large. In the oceans, every second  $10^{23}$  viral infections take place.

### **Objectives**

To fully comprehend the impact marine viruses can have on their host population dynamics and subsequently biodiversity, ecosystem functioning and global energy & matter fluxes, it is essential to study virus-host interactions under different environmental conditions.

### **Methods**

Here I will present experimental results on physiochemical variables affecting algal virus growth characteristics, as well as field data showing how a changing ocean due to global warming can control the magnitude of viral lysis as compared to the traditional loss factor, grazing.

### **Conclusions**

These examples illustrate the importance of environmental factors underlying the ecological importance of viral infection in marine ecosystems. Further research is warranted to identify more regulatory factors and understand the mechanisms by which they influence virus-host interactions.

**FEMS-0927**

**Viral infections and host**

**RECOMBINANT LIPIDATED DENGUE ENVELOPE PROTEIN DOMAIN III INDUCES DURABLE NEUTRALIZING ANTIBODY RESPONSES AND PROTECTIVE IMMUNITY**

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**Background**

The immunogen and immunopotentiator are two important components of vaccines. Combination of these two components into a single construct will have a great potential to increase the reactivity of immunogen. The lipid moiety of the recombinant lipoprotein provides a danger signal to activate antigen-presenting cells via toll-like receptor 2, which may further enhance immune responses.

**Objectives**

We selected dengue-4 envelope protein domain III (LD4ED III) as the immunogen and converted this candidate in lipidated form in *Escherichia coli*-based system. In the present study, the immunogenicity and protective immunity of LD4ED III in mice were evaluated.

**Methods**

Recombinant proteins were purified by immobilized metal affinity chromatograph columns. Groups of mice were immunized subcutaneously twice with 10 µg of D4ED III or LD4ED III in PBS at a 4-week interval. Mice immunized with PBS alone served as controls. Immune responses in mice were examined. All animal studies were approved and performed in compliance with the guidelines of the Animal Committee of the National Health Research Institutes.

**Conclusions**

The LD4ED III expressed in *E. coli* retains the proper conformation, which has the capability to compete with dengue virus for cellular binding sites. In addition, LD4ED III is more immunogenic than D4ED III to induce immune responses. Most importantly, LD4ED III elicits a durable neutralizing antibody response and inhibits viremia in LD4ED III vaccinated mice in the absence of exogenous adjuvant. These results suggest that lipidated dengue envelope protein domain IIIs are potential dengue vaccine candidates.

**FEMS-1352**

**Viral infections and host**

**THE ROLE OF ADENOVIRUS 36 AS A RISK FACTOR IN OBESITY: THE FIRST CLINICAL STUDY IN THE ADIPOSE TISSUE SAMPLES OF ADULTS IN TURKEY**

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**Background**

Obesity which develops due to multifactorial reasons, was associated recently with human Adenovirus-36 (Ad-36).

**Objectives**

The aim of this study was to investigate the prevalence of Ad-36 antibodies in obese adults and also investigate the DNA of Ad-36 in their adipose tissue.

**Methods**

In this cross-sectional and case-control based study, 49 obese adults (BMI  $\geq 30$  kg/m<sup>2</sup>) and 49 non-obese adults (BMI  $\leq 25$  kg/m<sup>2</sup>) were included in this study as patient and control groups, respectively. Adipose tissue samples obtained by the lipoaspiration method, were studied by PCR methods. Simultaneously, the peresence of Ad-36 antibodies and serum leptin and adiponectin levels were assessed by serum neutralization assay (SNA) and ELISA, respectively.

## **Conclusions**

Ad-36 antibody was detected in 6 (12.2%) of 49 patients by SNA and was statistically significant ( $p < 0.05$ ). We couldn't detect Ad-36 DNA in adipose tissue; however, we detected significantly higher Ad-36 antibody levels in the obese group compared to the non-obese group. Mean BMI and leptin levels were higher in the Ad-36 positive group, while adiponectin levels were found to be lower in the Ad-36 positive group. Although no statistically significant difference was found in cholesterol and triglyceride levels between the two groups ( $p > 0.05$ ), lower mean serum cholesterol and triglyceride levels were found in the Ad-36 positive patients. There is a need for extended serial, particularly cohort and human-based, studies in order to have a clear understanding of the Ad-36-obesity relationship.

**FEMS-2907**

**Viral infections and host**

## **PREVALENCE OF ASTROVIRUS IN FECAL SPECIMENS OF CHILDREN WITH GASTROENTERITIS**

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### **Background**

Norovirus genogroup I (NV GI) and NV GII sapovirus (SaV) genogroups I, II, IV, and V, human rotavirus A(HRV), adenovirus serotypes 40 and 41 (AdV), and human astrovirus (AstV) are the most common causes of viral gastroenteritis and result in large outbreaks of viral diarrhea. An estimated 1.8 million children die each year from largely preventable enteric illnesses, with the large majority of these mortalities occurring in developing countries. Gastroenteritis is a major cause of morbidity and mortality in children worldwide.

### **Objectives**

Astroviruses are one of the major viral pathogens responsible for gastroenteritis. In this study performs to determine the prevalence of astrovirus in fecal specimens of children with gastroenteritis.

### **Methods**

In this study 2490 stool samples from children less than five years with gastroenteritis from five cities of Iran were collected. The prevalence of human astroviruses was tested in patients with acute gastroenteritis by using conventional duplex reverse transcription (RT) -PCR and electrophoresis.

### **Conclusions**

Young children were much more likely to be admitted with acute gastroenteritis. In this study astrovirus was detected in 1.6% of samples. There is no relation between age, sex and type of virus. rotavirus was detected in 50% of astrovirus positive samples. rotavirus was detected with ELISA. astroviruses are an important viral agents of gastroenteritis in Iran. Because of co-infection of astrovirus, improved personal hygiene and rotavirus vaccination will reduce the incidence of co-infections.



**FEMS-0868**

**Viral infections and host**

## **DEAMINASE ACTIVITY OF APOBEC3 IS REQUIRED FOR EFFICIENT RESTRICTION OF PORCINE ENDOGENOUS RETROVIRUS**

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### **Background**

Porcine endogenous retroviruses (PERVs) present a unique concern associated with xenotransplantation because they have been shown to infect certain human cells *in vitro*. Human APOBEC3G (huA3G) is a single-strand DNA cytosine deaminase, which inactivates the coding capacity of the virus by deamination of cDNA cytosines to uracils. This reaction occurs within (-) DNA strand during reverse transcription, resulting in G-to-A mutation in the (+) strand.

### **Objectives**

A recent report showed that huA3G also could inhibit viral replication by cytidine deaminase-independent mechanisms. Two contrasting results with regard to huA3G-dependent inhibition of PERV transmission have been reported. Some group suggests that the mechanism of inhibition does not require the DNA deaminase activity of huA3G. In contrast, other group report that PERV restriction is attributed to cytidine deamination.

### **Methods**

To determine whether DNA deamination is required for huA3G-dependent inhibition of PERV transmission, we established a new set of 293-PERV-PK-CIRCE (human 293 cells infected with PK15-derived PERVs) clones stably expressing huA3G. In addition to huA3G, we included porcine APOBEC3G(poA3Z2-Z3) and murine APOBEC3(mA3). 293T cells were infected with virus-containing supernatant from 293-PERV-PK-CIRCE clones expressing huA3G, poA3Z2-Z3 and mA3. To search for antiviral mechanism of APOBEC3, genomic DNA from 293T cells was prepared at 10h postinfection and the PERV gag gene from 3D-PCR(differential DNA denaturation PCR) product was sequenced.

### **Conclusions**

huA3G, poA3Z2-Z3 and mA3 induced G-to-A hypermutations in the PERV genome effectively. Based on these results, we conclude that the APOBEC3 restricts PERV replication by deaminase-dependent mechanism.



**FEMS-1354**

**Viral infections and host**

**IS THERE ANY ROLE OF ADENOVIRUS 36 AS A RISK FACTOR IN GYNECOMASTIA ? THE FIRST CLINICAL STUDY WORLDWIDE FOR PATIENTS WITH GYNECOMASTIA**

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**Background**

Gynecomastia was indicated as a diseases which develops due to multifactorial causes.

**Objectives**

Based on adipose tissue affinity of Ad-36, we hypothesized, Ad-36 may also play a role in the development of gynecomastia by possibly accompanying increased regional adiposity.

**Methods**

In this cross-sectional and case-control based study, 33 adult male (BMI  $\leq$  25 kg/m<sup>2</sup>) and 15 adult male (BMI  $\leq$  25 kg/m<sup>2</sup>) with anatomic disorders without gynecomastia pathology were included in this study as patient group (PG) and patient control group (PCG), respectively. Breast reduction samples obtained by the lipoaspiration/subcutaneous mastectomy method, were studied by PCR methods.

Simultaneously, the presence of Ad-36 antibodies and serum leptin and adiponectin levels were assessed by serum neutralization assay (SNA) and ELISA, respectively.

## **Conclusions**

Ad-36 antibody was detected in 8 (24.2%) out of 33 PG by SNA and was statistically significant ( $p < 0.05$ ). Mean leptin levels were higher in the Ad-36 positive group, while adiponectin levels were found to be lower in the Ad-36 positive group. We couldn't detect Ad-36 DNA in breast reduction samples; however, we detected significantly higher Ad-36 antibody levels in the PG compared to the PCG. Ad-36 antibody positivity stayed in models in multivariate logistic regression analysis (with backward modeling) and has been identified as a risk factor. We report firstly Ad-36 may be a risk factor in gynecomastia. Moreover, with regard to leptin, adiponectin and serum cholesterol levels, our results suggest that there might be a relationship between Ad-36 and gynecomastia.

**FEMS-2735**

**Viral infections and host**

**THERAPEUTIC POTENTIAL OF A NEW KLEBSIELLA PNEUMONIAE BACTERIOPHAGE: ISOLATION, GENETIC CHARACTERIZATION AND PHAGE DEVELOPMENT IN SLOWLY GROWING BACTERIA**

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**Background**

*Klebsiella pneumoniae* is a facultative anaerobic Gram-negative bacterium associated with pyoderma in animals. *K. pneumoniae* can acquire resistance to carbapenems, thus becoming insensitive to almost all available antimicrobial agents. Phage therapy has the potential to replace antibiotic treatment. Lytic bacteriophages and/or their gene products such as lysins, can be easily used as therapeutic agents against bacteria as they are host specific and generally show no side effects.

**Objectives**

We focused on isolation and characterization of new bacteriophages against *Klebsiella pneumoniae*, which can be potentially used in phage therapy in animals.

**Methods**

We collected *K. pneumoniae* swab samples from the infected animals that could not have been treated with a standard antibiotic therapy. Afterwards, new lytic phage PRA33 was isolated from sewage and, with the use of electron microscopy analysis, it was structurally defined as a member of the *Siphoviridae* family. Phage plaque morphology analysis (the plaque clarity and the ability to increase its diameter in time) and determination of phage host range were carried out. Then, analysis of kinetics of adsorption and one-step growth curves were determined in bacteria growing with slow growth rates in a chemostat system. We also performed phage stability tests in various liquid media. Phage genome was sequenced *de novo* and the identification of putative ORFs was undertaken.

**Conclusions**

Based on the results of our experiments, we propose PRA33 phage to be a promising candidate for application in phage therapy against *K. pneumoniae* causing infections in animals that are otherwise difficult to treat.

**FEMS-2306**

**Viral infections and host**

## **DEVELOPMENT AND EVALUATION OF A SEROLOGICAL CHIKUNGUNYA ANTIBODY DETECTION ASSAY**

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### **Background**

Chikungunya (also named breakbone fever) is a highly emerging disease in many tropical settings with great socioeconomic impact. Chikungunya-Viruses are transmitted to humans by bloodsucking mosquitoes (*Aedes aegypti*, *Aedes albopictus*). The symptoms of Chikungunya include fever which can reach 39°C (102,2°F) a petechial or maculopapular rash usually involving the limbs and trunk, and arthralgia or arthritis affecting multiple joints which can be debilitating. The fever typically lasts for two days and abruptly comes down. Alphaviruses can be noticed as import or travel associated infection.

### **Objectives**

The aim of this work was to develop an serological assay to detect IgG and IgM antibodies against Chikungunya and to evaluate in endemic outbreak settings.

### **Methods**

An IgG-capture and IgM-capture ELISA was developed. Both take advantage of native antigens produced with a proprietary technique. In house measurements as well as external evaluations in many endemic regions of the world conducted by well know tropical institutes revealed excellent clinical sensitivity and specificity as well as high positive and negative predictive values. Data from the current outbreak in the Caribbean will be discussed.

### **Conclusions**

The newly developed ELISA seems to be a superior tool to diagnose past and acute Chikungunya infection in common and outbreak settings all over the world. It will assist diagnosis of travel returners with unknown fever as well as military in endemic operation area. To further improve Chikungunya diagnostic a Lineblot is currently under development as tool for conformation of ELISA results as well as for small labs with limited lab equipment.





**FEMS-1562**

**Viral infections and host**

**VACCINE-TYPE ATTENUATING MUTATIONS IN HIGHLY PASSAGED STRAIN OF VARICELLA ZOSTER VIRUS**

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Background: Varicella-zoster virus (VZV) is causative agent for chickenpox and zoster. Live attenuated vaccines have been developed based on Japanese Oka strain and Korean MAV/06 strain. Comparison of the complete sequences between vaccine and wild-type strains suggested at least 24 mutations important for attenuation in vaccine strains. Certain strains with high in vitro passage history such as the strains Ellen and 32p72 contained some of the vaccine-type mutations. Objective: In this study, we attempted to understand vaccine-type attenuating mutations in highly passaged VZV strains.

Methods: Genome sequences of clinical strains with different passages were determined by next-generation sequencing and compared with those of other VZV strains.

Conclusion: Vaccine-type mutations were found at position 106262 (T→C) in the Korean clinical strain YC01 passage 32 and at positions 560 (T→C), 105169 (A→G), 106262 (T→C) and 107252 (T→C) in YC01 passage 61. Similar mutations were also found in high-passaged another clinical strain YC02 at positions 560, 106262 and 107252. Same mutations at 106262 and 107252 were found in the strains Ellen and 32p72. Thus, in the course of in vitro passaging of VZV, mutations at 106262 and 107252 seemed to occur first, and followed by mutation at 560. Direct PCR sequencing of these positions in YC01 and YC02 strains at various passages identified the passage numbers when the attenuating mutations occurred. Further studies of in vitro passaging under attenuating conditions such as low temperature or guinea pig cells will help to understand the mechanism of attenuating mutations in VZV.

**FEMS-0929**

**Viral infections and host**

**LIPIDATED DENGUE-2 ENVELOPE PROTEIN DOMAIN III INDEPENDENTLY STIMULATES LONG-LASTING NEUTRALIZING ANTIBODIES AND REDUCES THE RISK OF ANTIBODY-DEPENDENT ENHANCEMENT**

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**Background**

Dengue virus is a mosquito-transmitted virus that can cause self-limiting dengue fever, severe life-threatening dengue hemorrhagic fever and dengue shock syndrome. The existence of four serotypes of dengue virus has complicated the development of an effective and safe dengue vaccine. Recently, a clinical phase 2b trial of Sanofi Pasteur's CYD tetravalent dengue vaccine revealed that the vaccine did not confer full protection against dengue-2 virus. New approaches to dengue vaccine development are urgently needed. Our approach represents a promising method of dengue vaccine development and may even complement the deficiencies of the CYD tetravalent dengue vaccine.

**Objectives**

To develop a vaccine with long-lasting neutralizing antibodies and reduces the risk of antibody-dependent enhancement

**Methods**

Two important components of a vaccine, the immunogen and immunopotentiator, were combined into a single construct to generate a new generation of vaccines. We selected dengue-2 envelope protein domain III (D2ED III) as the immunogen and expressed this protein in lipidated form in *Escherichia coli*, yielding an immunogen with intrinsic immunopotential activity.

**Conclusions**

The formulation containing lipidated D2ED III (LD2ED III) in the absence of exogenous adjuvant elicited higher D2ED III-specific antibody responses than those obtained from its nonlipidated counterpart, D2ED III, and dengue-2 virus. In addition, the avidity and neutralizing capacity of the antibodies induced by LD2ED III were higher than those elicited by D2ED III and dengue-2 virus. Importantly, we showed that after lipidation, the subunit candidate LD2ED III exhibited increased immunogenicity while reducing the potential risk of antibody-dependent enhancement of infection in mice.

**FEMS-0931**

**Viral infections and host**

**NANOPARTICLES-BASED VACCINE INDUCES PROTECTIVE IMMUNITY AGAINST EV71 INFECTION IN TOLL-LIKE RECEPTOR 9 KNOCKOUT MOUSE ANIMAL MODEL**

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**Background:**

Enterovirus 71 (EV71), a positive-stranded RNA virus, is the major cause of hand, foot and mouth disease (HFMD) with severe neurological symptoms. Seven-day-old toll-like receptor 9 knockout (TLR9KO) mice infected with a non-mouse adapted EV71 strain developed neurological lesion-related symptoms, including hindlimb paralysis, slowness, ataxia and lethargy. Histopathological examination revealed a massive damage in the limb muscles, brain, and spinal cord. The TLR9KO mouse could be useful as EV71 animal model to study anti-EV71 drugs and vaccines.

**Objectives:**

A novel nanoparticle-based EV71 VP1 peptide vaccine was evaluated in the EV71 mouse model. The clinical symptoms of mice after EV71 infection were monitored

**Methods:**

Seven-day-old TLR9KO mice were infected with non-mouse adaptive EV71 and showed neurological diseases. We found that EV71 alone cannot activate toll-like receptor 9 (TLR9) signaling. On the contrast, supernatant from EV71-infected cells could activate TLR9. Furthermore, we identified the EV71-infected cells-derived danger signal activate TLR9 to protect mice from EV71 challenge. To investigate whether TLR9KO mice could be used as an animal model for EV71 vaccine development, EV71-infected TLR9KO mice were treated with sera from vaccinated mice. We designed a VP1 peptide of EV71 that formulated with emulsion type nanoparticles adjuvant and immunization with wild type mice. Sera from immunized mice were administered to EV71-infected TLR9KO mice, and clinical neurological symptoms of mice were observed.

**Conclusions:**

Sera from EV71 VP1 peptide immunized mice could reduce neurological symptoms of TLR9KO mice from challenged with EV71.



**FEMS-1230**

**Viral infections and host**

**ALGAL VIRUS PRODUCTION IS NEGATIVELY AFFECTED BY PHOSPHORUS AND NITROGEN LIMITATION, BUT THIS MAY BE COUNTERACTED BY NUTRIENT ASSIMILATION DURING INFECTION**

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**Background**

Viruses are a main cause of phytoplankton mortality, thereby controlling their community composition and driving biogeochemical cycling in the marine environment. Phytoplankton growth and production is often limited by nitrogen (N) and/ or phosphorus (P), which can affect the virus growth characteristics upon infection. The influence of nutrient limitation on phytoplankton – virus interactions are, however, still poorly studied. As climate change will lead to an expansion of stratified and consequently nutrient limited oceanic regions, it is important to understand how phytoplankton mortality rates in the future marine environment will be affected by these changes.

**Objectives**

Our goal was to study the effects of N- and P-limitation on virus host interaction and the potential relieve of these effects by the uptake of nutrients during viral infection.

**Methods**

Therefore, cultures of the the phytoplankton species *Micromonas pusilla* and *Phaeocystis globosa* were grown semicontinuously under limitation of either P, N, or NP until the moment of infection. Upon infection the abundances of host cells and viruses were monitored to obtain one-step growth curves.

**Conclusions**

Nutrient limitation led to elongated viral latent periods (up to 3-fold) and strongly reduced (up to 8-fold) burst sizes, whereby the outcome was dependent on both the species and the limiting nutrient involved. Furthermore, the negative effects of P-limitation on virus production in the picophytoplankter *M. pusilla* could be overcome by spiking the infected and P-limited cultures with small pulses of inorganic and organic P, simulating bacterial remineralization and supply by lysis of neighboring cells, respectively.

**FEMS-0508**

**Viral infections and host**

**SAFFOLD VIRUSES IN PEDIATRIC PATIENTS WITH DIARRHEA IN THAILAND**

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**Background**

In recent years, several new viruses associated with diarrheal diseases in human have been reported. Saffold virus (SAFV) is one of a newly identified human Cardiovirus reported for the first time in 2007.

**Objectives**

This study investigated the prevalence of SAFV and characterized the genotypes distributed in children with diarrhea in Chiang Mai, Thailand during 2012-2013.

**Methods**

A total of 608 fecal specimens collected during January 2012 to December 2013 from children with diarrhea in Chiang Mai, Thailand were investigated for SAFV by RT-nested PCR and performed sequence analysis.

**Conclusions**

Nine out of 608 (1.5%) were positive for 4 genotypes of SAFV, SAFV1, SAFV2, SAFV3, and SAFV4. SAFV mono-infection was found in 5 cases, while co-infection with other viruses that cause diarrhea was observed in 4 cases. This study provides more information about SAFV genotypes circulating in pediatric patients with diarrhea in Thailand.

**FEMS-2782**

**Viral infections and host**

**THE CYTOKINES EXPRESSION IS MODULATED BY EPIGENETIC MECHANISMS  
IN RESPONSE TO INFECTION WITH IPNV**

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**Background**

The massive salmon farming associated to aquaculture industry has brought a significant increase in infectious diseases, thereby generating economic losses. The infectious pancreatic necrosis virus (IPNV), agent of high mortalities in fish farmed, has capacity to activate the immune response mediated by interferon in their host; the aim of this study is clarify whether the activation of the antiviral response caused by IPNV is through epigenetic mechanisms.

**Objectives**

We observed that expression of cytokines, in salmonids cells infected with IPNV was associated to promoter methylation status. Moreover, DNA methyltransferase inhibitors treatments were able to modulate the expression of these cytokines.

**Methods**

Studies of methylation in CpG islands of the promoters of cytokines in infected salmonids cells with IPNV were conducted. Through bisulfite sequencing, change in methylation of the promoter affecting the expression of interferon was evaluated. Furthermore, treatments with DNA methyltransferase (DNMT) inhibitor 5-azacytidine and 5-Aza-2'-deoxycytidine were performed to evaluate the expression of cytokines.

**Conclusions**

These results show that cytokines expression is regulated by promoter methylation status which could be correlated with the modulation of DNMT activity when cells are infected with IPNV. Our work suggested that epigenetic mechanisms could participate in the regulation of host genes expression in IPNV infection, and provided a new insight into understanding the mechanisms of viral infection.

**FEMS-2475**

**Viral infections and host**

**EPIDEMIOLOGICAL, CLINICAL MANIFESTATION AND GENOTYPE CHARACTERIZATION OF HEPATITIS-C VIRUS AMONG THE HIV PATIENTS IN KASHAN**

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**Background**

Due to shared transmission routes, HCV infection is highly prevalent among people infected with human immunodeficiency virus (HIV). Little is known about the characteristics of HCV genotypes in among HIV/HCV coinfecting patients in Kashan

**Objectives**

In order to provide valuable information for HCV management in this particular population, we investigated the HCV genotypes in HIV-infected In Kashan-Iran.

**Methods**

This study was conducted in 2013 in kashan, Iran. The study population consisted of all HIV infected having records in behavioural counselling center and prison in Kashan . Demographic information and HCV, HIV-related risk behaviours were obtained through an interviewer-assisted questionnaire. After taking consent form, 10 cc blood taken from HIV patients and serum samples were screened for HCV infection using enzyme-linked immunosorbent assay (ELISA), and detection of HCV RNA was performed by PCR amplification. HCV subtypes were determined by direct sequencing of amplicons

**Conclusions**

54(85%) out of the 63 HIV infected patients , were HCV positive which all of them were male..there was a significant correlation between HCV frequency and occupation ( $P<0.0001$ ) and level of education ( $p<0/05$ ). 100% of HIV/HCV coinfecting cases had history of illicit drug use,92.6% history of prison and and 40.7% had high risk sexual behavior. There was found genotype 1 in 75.9% and genotype 3 in 24.1% of HCV patients .94.4% of HCV patients had subtype a. The mean of AST,ALT,TLC,CD4 in HCV patients were 50.6,58.5,397.2,398.8. There was no clinical symptom of chronic Hepatitis c.

The majority of HIV infected persons in Kashan city are HCV positive and predominant genotype is 1 and predominant subtype is a.



**FEMS-2653**

**Viral infections and host**

## **PREVALENCE OF HEPATITIS C IN INJECTING DRUG USERS IN KASHAN -IRAN**

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### **Background**

Drug injection is a very important risk factor for viral hepatitis and human immunodeficiency virus (HIV) infection

### **Objectives**

The present study was performed to evaluate the prevalence of hepatitis C among injection drug users (IDUs) and to identify the related risk factors for these infections in this group.

### **Methods**

This descriptive-analytical study was conducted in 2011 in kashan, Iran. The study population consisted of 300 IDUs in MMT, DIC and counselling centers. Demographic information and HCV related risk behaviors were obtained through an interviewer-assisted questionnaire. IDUs serum samples were screened HCV infection using enzyme-linked immunosorbent assay (ELISA). Data analyzed using Spss.

### **Conclusions**

Of the 300 IDUs, 288 (96%) were male. The majority of IDUs 127 (42.3%) were in 30-39 age group with mean age  $34.9 \pm 9.7$ . The majority of IDUs 224 (74.7%) had more than 10 years history of addiction. The most common age of onset addiction was 15-20 year 134 (44.7%). The prevalence of HCV was 142 (47.3%). It was found that there was a significant correlation between using shared syringe, age and times of prison and HCV infection.

There was high prevalence of HCV among IDU. High risk behaviors such as tattooing, unsafe sex, needle sharing are common so regular screening of IDU, education of personal Health about using sterile syringe, HBV vaccination and treatment of addiction and HCV infection is recommended.

**FEMS-0823**

**Viral infections and host**

**DETECTION AND PHYLOGENETIC ANALYSIS OF TORQUE TENO VIRUS (TTV) AMONG BLOOD DONORS AND HBC OR HBV-INFECTED PATIENTS IN QATAR**

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**Background**

TTV has been linked to non A-G hepatitis

**Objectives**

The aim of this project was to investigate the rate of infection and to determine the genotypic features of TTV in Qatar

**Methods**

A total of 644 blood samples representing different nationalities, non-Qatari (526) and Qatari (118) nationals (mostly from Arab and South East Asia countries) were studied for the presence of TTV DNA by nested PCR. The majority (573) of the blood samples belonged to healthy blood donors, while 53 and 54 of the blood samples belonged to hepatitis C virus (HCV) and hepatitis B virus (HCV)-infected patients, respectively.

**Conclusions**

**Results:** we showed that the infection rates of TTV in blood donors, and those HCV or HBV-infected patients were 81.4%, 84.9% and 90.75%, respectively. Significant association found between TTV viremia and nationality, or age was detected. Sequence analysis of PCR fragments amplified from the 5'-untranslated region (5'-UTR) of all (531) TTV positive samples showed that 65.5% (348/531) of the PCR fragment sequences were classified into main genotype 3, followed by genotype 5 (24%), 2 (5.8%) and 1 (4.7%). Interestingly, genotype 4 was not detected among the studied subjects. Phylogenetic and pairwise analyses using sequences from TTV viremic samples also showed an overall close similarity to the main genotype 3. **In conclusion**, there was no significant difference in the detection rates of TTV among Qatari nationals and non-nationals, and most TTV genotypes, particularly genotype 3, were detected

**FEMS-2940**

**Viral infections and host**

**PARTIAL CHARACTERIZATION OF A PROBABLE NOVEL SUBTYPE OF  
BETAPAPILLOMAVIRUS HPV 17**

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**Background**

Human papillomavirus (HPV) have epithelial and mucosal tropism. HPV type 17 belongs to cluster *b2* of *Betapillomavirus*. Distinct subtypes with dissimilarities of 2 to 10% at the nucleotide level from their reference HPV can be identified. Molecular cloning experiments enabled to recognize two subtypes (17a and 17b) isolated from epidermodysplasia verruciformis (EV)

**Objectives**

The aim of this study was investigate a possible novel subtype of *Betapapillomavirus* HPV type 17.

**Methods**

The isolate was collected from an oral mucosa of an asymptomatic woman. Partial L1 region was amplified using My09/11 degenerated primers. The complete E6, E7, and LCR genes were amplified with specific primers. The Ethics Committee of the College of Medicine at the University approved the protocols for collection and informed consent.

**Conclusions**

The viral DNA was sequenced and partially characterized. Within these sets, the DNA sequence was altered at 38 positions (15 in L1, 13 in E6, 8 in E7, and 2 in LCR gene). Partial L1 analysis showed high dissimilarity facing to prototype, reaching 5% of nucleotide substitutions. The E6 oncoprotein presented the highest modification among the sequences studied. The amino acid modified at position 62 (S-T) affected one zinc binding domain (CxxC(C)<sub>29</sub>CxxC) of this protein. E2 binding factors, transcription factors, and TATA signals were seen in LCR region. Despite the EV-associated HPV 17 has cutaneous tropism, the oral cavity is an appropriated niche to host HPV which naturally infects other sites. Based on these findings, we believe to have found a novel subtype of this type.

**FEMS-1090**

**Viral infections and host**

## **IMMUNOBIOTICS MODULATE ANTIVIRAL IMMUNITY AGAINST ROTAVIRUS VIA TOLL-LIKE RECEPTORS PATHWAYS IN PORCINE INTESTINAL EPITHELIAL CELLS**

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### **Background**

Rotavirus (RV) is a primary cause of acute diarrhea in infants and young animals, and despite attenuated RV vaccines have been able to reduce the global disease burden associated with RV gastroenteritis, this infectious disease is still a problem in developing countries. Several reports indicate that immunobiotics have the ability to reduce severity of RV mediated diarrhea. Previously, we demonstrated that some immunobiotic strains were able to beneficially modulate Toll-like receptor (TLR)-3 mediated immune response in porcine intestinal epithelial (PIE) cells.

### **Objectives**

To evaluate the capacity of immunobiotics to modulate RV-induced antiviral response in PIE cells.

### **Methods**

(i) PIE and MA104 cells were inoculated with several strains of RVs to investigate the susceptibility and the ability to induce the inflammatory cytokines against RV infection, (ii) The capacity of immunobiotics to beneficially modulate RV-induced immune response in PIE cells was investigated, by evaluating inflammatory cytokines, type I interferons, TLR signaling pathways, and TLR negative regulators.

### **Conclusions**

Challenge of PIE cells with RV reduced cell viability and increased the expression of IFN- $\beta$ , MCP-1, IL-6, IL-8, and TLR3. Immunobiotics were able to improve cell viability, and to reduce RV-induced inflammatory cytokines expression. Those effects were related to the capacity to modulate TLR negative regulators single such as SIGIRR, Tollip, A20, Bcl-3, IRAK-M, and MKP-1. Our results demonstrated that immunobiotics can be used as immunomodulators to alleviate viral induced diarrhea in livestock animals such as the pig.



**FEMS-1505**

**Viral infections and host**

**NON-VIABLE IMMUNOBIOTIC LACTOBACILLUS RHAMNOSUS CRL1505 IMPROVES RESPIRATORY IMMUNITY AND REDUCES INFLUENZA VIRUS-ASSOCIATED LUNG INJURY**

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**Background**

Symptoms of influenza infection are variable ranging from mild respiratory distress to massive organ failure resulting in death. Seasonal influenza is usually self-limiting but in susceptible patients it may progress to acute lung injury, which is characterized by augmented pulmonary microvascular permeability, hypoxemia and respiratory failure. The ongoing threat of influenza epidemics and pandemics has emphasized the importance of developing novel, safe and effective therapeutic or preventive strategies against infections from divergent influenza viruses.

**Objectives**

To examine the effect of nasal administration of heat-killed *Lactobacillus rhamnosus* 1505 (Lr1505) on immune response to influenza infection in mice.

**Methods**

Six-week old BALB/c mice were treated with  $10^8$  cells of Lr1505 by the nasal route during two consecutive days. Lr1505-treated and untreated control mice were then nasally challenged with Influenza virus. Lung tissue damage and respiratory and systemic immune responses were studied at several time points after viral challenge. Results demonstrated that non-viable immunobiotic strain protected infected mice by reducing pulmonary injury and lung viral loads through several mechanisms: a) inflammatory cytokines were down-regulated diminishing inflammation ( $p < 0.01$ ), b) IFNs with direct antiviral activity were enhanced ( $p < 0.01$ ), c) regulatory cytokines IL-10 was up-regulated ( $p < 0.05$ ), d) maturation markers and co-stimulatory molecules were up-regulated in lung antigen presenting cells ( $p < 0.01$ ), e) IFN- $\gamma$  and IL-10 producing CD4<sup>+</sup> lymphocytes were recruited to the lungs.

**Conclusions**

Our results strongly suggest that administration of Lr1505 may represent an interesting alternative to modulate respiratory immune response and reduce influenza

virus-associated pulmonary damage.

FEMS-1807

Viral infections and host

# POPULATION DYNAMICS OF A CYANOBACTERIUM MICROCYSTIS AERUGINOSA USING AMPLICON ANALYSIS FOR THE LEADER-END CRISPR FRAGMENTS

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**Background.** Freshwater cyanobacterium *Microcystis aeruginosa* frequently forms massive natural blooms where the cell densities are comparable to those in laboratory media ( $10^7$  to  $10^8$  cells/ml). The representative genome of this species contains abundant and diverse potential anti-viral defense genes. Our culture analyses showed distinct CRISPR arrays in this species. This suggested the *Microcystis* cyanophage may have co-evolved with its phage in a frequency-dependent selection.

**Objectives.** The aim of this study is to understand the co-evolutionary dynamics of the *Microcystis* cyanophage in natural settings.

**Methods.** The CRISPR diversity of *Microcystis aeruginosa* was examined in a small eutrophic pond using amplicon analysis of the leader-end of CRISPR fragments with a MiSeq mini-gene sequencer.

**Conclusions.** During the sampling period, a CRISPR-related genotype (CT19) occurred frequently in the *Microcystis* population. A qPCR targeting CT19 showed the copy numbers were from  $4.9 \times 10^2$  and  $3.2 \times 10^5$  copies  $\text{mL}^{-1}$  and accounted for 0.2 % to 18 % of the *Microcystis* population. We obtained 176,800 leader-end CRISPR fragments using amplicon sequencing. Of these, 116,466 were reclassified into CT19 consisting of two major variants (28% and 72%, respectively). These major CTs co-existed and their numbers oscillated throughout the sampling periods. A few new variants were found to acquire new spacers or the lack of spacers at the leader-end of the major variants. This indicates *Microcystis* cyanophage co-evolution creates a few new genetic variations and also maintains the genetic diversity in the population.



**FEMS-1144**

**Viral infections and host**

**PRODUCTION IN YEAST OF HUMAN BOCAVIRUS 1-4 VP2 VIRUS-LIKE PARTICLES AND GENERATION OF VP2-SPECIFIC MONOCLONAL ANTIBODIES AS NOVEL TOOLS FOR BOCAVIRUS SEROLOGY**

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**Background**

Human bocavirus 1 (HBoV1), first described in 2005, was considered a causative agent of previously unexplained respiratory tract diseases. Recently, 3 new members of genus *Bocavirus*, HBoV2-4 were described. HBoV2-4 occurs mainly in the gastrointestinal tract but rarely in the respiratory tract, contrary to HBoV1.

**Objectives**

Recombinant viral antigens have been proven useful for serologic diagnosis of viral infections. Production of HBoV1-4 antigens in yeast expression system has not yet been reported. In the current study, the capsid proteins VP2 of HBoV1 - 4 were expressed in yeast *S.cerevisiae*. Electron microscopy demonstrated that all purified recombinant proteins self assembled into virus-like particles (VLPs) exhibiting the typical icosahedral appearance of parvoviruses with a diameter of approximately 20 nm. HBoV1 - 4 VP2 VLPs were stable in yeast and were easily purified by caesium chloride gradient ultracentrifugation. Four monoclonal antibodies (MAbs) of IgG1 subtype were generated by immunization of mouse with recombinant HBoV1 VP2 VLPs. Three of them specifically recognized only HBoV1 VP2 protein; one MAb was cross-reactive with HBoV2 and HBoV4 VP2 proteins. Recombinant HBoV1-4 VP2 VLPs and VP2-specific MAbs were employed to develop serological assays to detect virus-specific IgG antibodies in human serum specimens.

**Methods**

Cloning of HBoV VP2 proteins coding sequences into yeast vectors and purification of virus-like particles from yeast. Electron microscopy. SDS-PAGE and Western blotting analysis. Indirect ELISA. IgG capture ELISA. Generation of monoclonal antibodies.

**Conclusions**

Yeast expression system proved to be simple, efficient and cost-effective, suitable for high-level production of HBoV1- 4 VP2 as VLPs, that resemble native virus in regards of morphology and antigenicity.

**FEMS-2787**

**Viral infections and host**

## **REMARKABLE DIVERSITY AMONG NOVEL DENSOVIRUSES FROM CRICKETS**

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### **Background**

Densoviruses are members of the Parvoviridae family infecting invertebrates. Since 2009, a cricket densovirus, AdDV, caused a severe epidemic in the \$ 600-million cricket industry in North-America.

### **Objectives**

During the last 18 months, we also received samples of diseased crickets from North America, Europe and Japan that were negative for AdDV. Electron microscopy demonstrated that these samples contained densovirus-like particles. Objectives of this study included characterization of these virus(es), cloning, sequencing, and X-ray crystallography.

### **Methods**

A SISPA method was used to detect the pathogens (J Virol 88:12152). Sequencing of the amplicons identified those with identities to known viruses allowing by primer extension to obtain the complete genomes and cloning in suitable vectors to obtain infectious clones. Sequencing revealed that some were novel circoviruses and a novel ambisense densovirus (Genome Announc. 1(2):e00079-13, 1(3):e00328 and 131(6):e00914-13). However, the greatest surprise was a densovirus with a segmented genome (AdSDV). NS- and VP- coding sequences are in separate segments of about 3.3 kb. Both NS- and VP- segments are flanked by identical hairpin terminal sequences but lack ITRs. The two ORFs for VP arose through recombination between a mosquito brevidensovirus and AdDV. The downstream ORF with AdDV-VP1up-like phospholipase A2 could be spliced to the C-terminus of the major capsid protein. Currently, the capsid structure (X-ray crystallography) and transcription strategy of AdSDNV.

### **Conclusions**

A brevidensovirus that normally infects only mosquitoes has adapted itself through recombination with AdDV to crickets. These findings necessitate a revision of the definition and taxonomy of parvoviruses.

**FEMS-2207**

**Viral infections and host**

**TIME SINCE ONSET OF DISEASE AND INDIVIDUAL CLINICAL MARKERS  
ASSOCIATE WITH TRANSCRIPTIONAL CHANGES IN UNCOMPLICATED  
DENGUE**

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**Background**

Dengue virus (DENV) infection causes viral haemorrhagic fever that is characterized by extensive activation of the immune system.

**Objectives**

The aim of this study is to investigate the kinetics of the transcriptome signature changes during the course of disease and the association of genes in these signatures with clinical parameters.

**Methods**

Sequential whole blood samples from DENV infected patients in Jakarta were profiled using affymetrix microarrays, which were analysed using principal component analysis, limma, gene set analysis, and weighted gene co-expression network analysis.

**Conclusions**

Time since onset of disease associates with the shift in transcriptome signatures from immunity and inflammation to cell cycle and repair mechanisms in patients with non-severe dengue. The strong association of time with blood transcriptome changes hampers both the discovery as well as the potential application of biomarkers in dengue. Clinical diagnosis (according to the WHO classification) does not associate with differential gene expression. However, network analysis did show that that key clinical markers, including platelet count, fibrinogen, albumin, IV fluid distributed per day and liver enzymes SGOT and SGPT, strongly correlate with gene modules that are enriched for genes involved in the immune response. The expression level of these gene modules may support earlier detection of disease progression as well as clinical management of dengue.

Ref: CAM van de Weg\*, HJ van den Ham\*, MA Bijl, F Anfasa, F Zaaraoui-Boutahar, BE Dewi, L Nainggolan, WFJ van IJcken, ADME Osterhaus, BEE Martina, ECM van Gorp, AC Andeweg. PLoS Neg Trop Dis 2015

**FEMS-2439**

**Virome**

## **COMPLEXITY OF THE VIROME FROM THE KY ISOLATE OF PHOMOPSIS LONGICOLLA FUNGUS**

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### **Background**

*Phomopsis longicolla* (teleomorph = *Diaporthe*) is an ascomycete phytopathogenic fungus. Mycoviruses were reported to debilitate their hosts [1]. The KY isolate and derived subcultures of *P. longicolla* exhibited abnormal growth and reduced virulence. Initial examination revealed that the isolate harbors *Phomopsis longicolla* hypovirus 1 (PIHV1) [2]. This ssRNA virus belongs to a group of betahypoviruses that, unlike alphahypoviruses, were not reported to reduce virulence of their hosts.

### **Objectives**

The objective of the study was to investigate potential viral (or other) determinants of hypovirulence and characterise structure and diversity of PIHV1 population in the KY isolate of *P. longicolla*.

### **Methods**

Illumina-based DNA and RNA deep sequencing was performed of a number of the KY subcultures and the type strain of *Phomopsis longicolla* Hobbs, anamorph (ATCC® 60325™).

### **Conclusions**

The analyses of the NGS data revealed that the virome of the KY isolate consists of PIHV1 recombinant with a previously undescribed hypovirus, population of deletion mutants of PIHV1, and a novel RNA virus related to members of *Narnaviridae* family.

**FEMS-1419**

**Virome**

## **CHARACTERIZING THE VIROME OF THE ENTOMOPATHOGENIC FUNGUS BEAUVERIA BASSIANA**

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### **Background**

Entomopathogenic fungi are of scientific interest because they enable analysis of virus-host interactions and can be used as biocontrol agents of insect pests. The deuteromycetous fungus *Beauveria bassiana* has a widespread geographical distribution and a wide host range. Mycoviruses have been described mostly in phytopathogenic fungi and are occasionally associated with hypovirulence, while in entomopathogenic fungi presence of mycoviruses has been reported only rarely.

### **Objectives**

The aim of the present study is to detect and analyse the viruses present in a collection of *B. bassiana* isolates sourced from worldwide locations.

### **Methods**

Population studies, RT-PCR, cloning and sequencing, hybridization experiments, time-course studies and transmission electron microscopy (TEM) were used to characterize the virome of *B. bassiana*.

### **Conclusions**

A population study revealed that 17/75 (22.7%) *B. bassiana* isolates harbor dsRNA elements. Two members of the *Partitiviridae* family, *B. bassiana* partitivirus-1 and -2 (BbPV-1 and BbPV-2), have been sequenced, while TEM revealed the presence of virus-like particles. Hybridization experiments revealed that BbPV-1 and BbPV-2 are present in seven *B. bassiana* isolates derived from different hosts and geographical origins. Additionally, four members of the *Totiviridae* family, originating from the Iberian Peninsula and the Canary Islands, have been partially sequenced. Furthermore, two previously uncharacterized viruses, *B. bassiana* polymycovirus-1 (BbPmV-1) and *B. bassiana* non-segmented virus-1 (BbNV-1), have been detected and fully sequenced and the prevalence of further PmV-like viruses in *B. bassiana* is currently under investigation. Finally, a time-course study revealed a strong negative

correlation between the copy numbers of viral dsRNA and the developmental stages of the fungus.

**FEMS-1398**  
**Free subjects**

**THE NEW MESOPHILIC FILAMENTOUS ANOXYGENIC PHOTOTROPHIC BACTERIUM FROM SULFIDE HOT SPRINGS.**

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**Background**

Filamentous anoxygenic phototrophic bacteria (FAPB) belong to the deep-branching phylum Chloroflexi, and are considered to be the most ancient lineage of phototrophs. The unambiguous reconstruction of the evolution of FAPB is complicated by poor representativeness of mesophilic FAPB.

**Objectives**

The above mentioned pointed our efforts to the search and study of new mesophilic FAPB in mesothermal cyanobacterial mats from hot springs in Barguzin valley (Buryat Republic, Russia).

**Methods**

Mat samples were collected from mesothermal zones of Umkhey (36 – 40°C) and Kuchiger (35 - 39°C) sulfide hot springs. The enrichment culture containing mesophilic green filamentous anaerobic bacterium Um-2 was obtained from Umkhey mat sample at the Pfenning medium (pH 7.0) at 28°C under illumination. The Um-2 cells (diameter about 0,8 µm) were combined to trichome. The main cellular pigments was bacteriochlorophyll *c* (maximum absorption at 740 nm). Gas vacuoles were not found. According to the phylogenetic test results (by 16S), Um-2 formed the branch belonging to mesophilic FAPB cluster. The nearest neighbor of Um-2 was “*Candidatus Chloroploca asiatica*” Um-3 (KJ605349) (91% of similarity). The Um-2 phylotype representatives was detected in Umkhey and Kuchiger mat samples using PCR test with FAPB-specific *puLM* primers. The sequences of *rrna* (16S rRNA coding) and *puLM* genes were deposited in GenBank under accessions KP341999 and KP342000 respectively.

**Conclusions**

Thus the enrichment culture of new mesophilic FAPB belonging to the new genus and species of the Chloroflexales order was obtained. It was shown that the new bacterium is presented in the microbial community of sulfide springs of Barguzin valley.



**FEMS-0578**  
**Free subjects**

#### **ANTIFUNGAL ACTIVITY OF NONHYDROLYTIC SYNTHESIZED TiO<sub>2</sub>**

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#### **Background**

Despite of many studies on antibacterial activity of titanium dioxide (TiO<sub>2</sub>), its antifungal properties are not yet well evaluated.

#### **Objectives**

The aim of our study is to exam the activity of nanosized TiO<sub>2</sub> against yeast cells.

#### **Methods**

TiO<sub>2</sub> used was synthesized by sol-gel method and characterized as anatase with particles size about 20 nm. Its antifungal activity was examined against *C.albicans*, *C.tropicalis*, *C.lusitaniae*, *C.glabrata* and *C.krusei*, using the following experimental setup: concentration of TiO<sub>2</sub> powder – 1 mg/ml, 100 ml fungal suspension with cell density about 10<sup>4</sup> CFU/ml and BLB lamp (50 Hz 8W T5 with emission 365 nm) situated at distance 10 cm. At regular intervals of time, samples were taken and serial dilutions were prepared. The number of viable cells was detected by spread plate method. For determination of the post-irradiation effect, the treated suspensions were tested after 48 h dark period.

#### **Conclusions**

The data obtained about antifungal activity of TiO<sub>2</sub> are in agreement with photocatalytic properties of metal oxide nanoparticles. After 120 min UV-A photocatalytic treatment with 1 mg/ml TiO<sub>2</sub>, the number of colonies of *C.albicans* and *C.tropicalis* decrease from about 10<sup>4</sup> CFU/ml to less than 5 CFU/ml with 100% reduction index. Final reduction index in *C.lusitaniae* and *C.glabrata* is 99.643% and 99.898%, respectively. These data indicate strong photocatalytic fungicidal effect of TiO<sub>2</sub> on the tested fungi. At the same conditions, the colonies of *C.krusei* are reduced with 98%. Our data show that the nonhydrolytic synthesized nanosized TiO<sub>2</sub> possesses antifungal activity against isolates of the genus *Candida* with some differences in the speed of photocatalytic process.



**FEMS-2722**  
**Free subjects**

### **CIPROFLOXACIN EFFECT ON SERUM OXALIC ACID LEVEL**

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### **Background**

Ciprofloxacin ranks among the primary drugs used as treatment in urinary system infections in adults.

It is known that *Oxalobacter formigenes* colonization in intestines is disrupted by antibiotic intake.

Due to the *Oxalobacter formigenes* colonization deficiency, the fact that high level serum oxalic acid

results in formation of renal calcium oxalate stones has been determined.

Additionally, serum oxalic

acid level increase is thought to have negative effect on urinary and cardiovascular system.

### **Objectives**

The purpose of our study is to search whether there is an increase in serum oxalic acid level because

of intestinal flora disorder in the patients with urinary system infection and the individuals given

ciprofloxacin as treatment.

### **Methods**

In our study, 40 people who have urinary system infection and take ciprofloxacin treatment have been included. Patients serum oxalic acid levels were evaluated both in pre-treatment and post-treatment evaluation which

was a month later. Serum oxalic acid was measured by a commercially available kit (SunRed Biological Technology, Serial number: 201-12-2153), using Human Oxalic Acid ELISA method.

### **Conclusions**

In conclusion, when pre-treatment and post-treatment serum oxalic acid levels were compared,

no statistical difference was found ( $p=0,47$ ). It was found that ciprofloxacin treatment didn't affect

serum oxalic acid levels of the patients with urinary system infection. In the light

of this data,  
it is concluded that these patients don't need to be followed up for any possible complications  
because of high level serum oxalic acid.

**FEMS-2511**  
**Free subjects**

**POTENTIAL SOURCES OF COMMUNITY CLOSTRIDIUM DIFFICILE  
INFECTIONS: OVERLAP OF PCR-RIBOTYPES ISOLATED FROM ANIMALS AND  
THE ENVIRONMENT**

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**Background**

Anaerobic bacterium *Clostridium difficile* has been traditionally considered as a human nosocomial enteropathogen. Although its natural habitat is human and animal intestine, the ability to form dormant endospores enables *C. difficile* to survive also in aerobic environment. Recent emergence of community acquired *C. difficile* infections and infections in animals implies that environment might be a reservoir of *C. difficile* and a source of infection.

**Objectives**

Our aim was to investigate diversity and overlap of *C. difficile* PCR-ribotypes isolated from animals and different non-hospital environments in Slovenia.

**Methods**

Strains included in the comparison originated from domestic animals (calves, piglets, horses, poultry and birds, goats, sheep, dogs and cats) and different environments (puddles and soil from rural and urban areas, compost and water from rivers and ponds) in Slovenia. Isolates were characterized by PCR-ribotyping and toxinotyping.

**Conclusions**

This work is unique in presenting a large number of *C. difficile* strains from several rural and urban environments and compare them to animal and human isolates. Altogether 80 PCR-ribotypes (47 in animals and 58 in the environment) were identified and 25 were shared between animals and the environment. Forty-six of

these PCR-ribotypes were found also in humans in Slovenia. Twenty-seven PCR-ribotypes were identified as non-toxigenic with more than half found only in the environment, mainly puddles and soil, which seem to represent an independent environment. Our results show that *C. difficile* genotypes that are commonly isolated from animals can also be isolated from different environments, indicating that non-hospital environment could represent an important reservoir.

**FEMS-1226**  
**Free subjects**

## **TRANSPORT OF EUKARYOTIC CELLS THROUGH ELECTROHYDRODYNAMIC STRUCTURES**

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### **Background**

When a high-voltage direct-current is applied to two beakers filled with water, a horizontal electrohydrodynamic (EHD) bridge forms between the two beakers. In this work we studied the transport and behavior of yeast cells and ThP1 human-monocytic-cell-line in this special environment.

### **Objectives**

The behavior of *Escherichia coli* in an EHD-bridge set-up was recently investigated: *E.coli* cells survive the procedure; the main direction of cell transport is from the cathode to the anode beaker. The goal of this work was to test in how far these results are specific for *E.coli*, thus studies with yeast cells and ThP1 cells were conducted.

### **Methods**

Cells were added to one or to both beakers, and the transport of the cells through the bridge was investigated using optical and microbiological techniques. One experimental series consisted of three different configurations: carrier solution in both beakers, only in the anode or only in the cathode beaker. As carrier solutions for yeast 5% glycerol and ThP1 cells 4.5% glucose were used.

### **Conclusions**

The behavior of cells in an EHD-bridge depends largely on their size and their surface charge. The heavier the organism, the slower the transport; the higher the surface charge, the more pronounced the preferred flow direction.

*S.cerevisiae*: transport was observed in both directions. The absence of a preferred flow direction means that electric forces play a smaller role which is corroborated by the zeta potential.

ThP 1 cells survive the transport and showed similar behavior as the *E.coli* cells: pronounced transport towards the anode.



**FEMS-0640**  
**Free subjects**

**POST-ANTIBIOTIC AND POST-ANTIBIOTIC SUB-MINIMAL INHIBITORY  
CONCENTRATION EFFECTS OF CHLORHEXIDINE AGAINST ORAL BACTERIA**

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**Background**

The post-antibiotic effect (PAE) is defined as the suppression of bacterial growth for a particular duration after a brief exposure to an antimicrobial agent. Chlorhexidine is a widely used biocide found in antiseptic products; however, its PAE remains unclear.

**Objectives**

In this study, PAE, post-antibiotic sub-minimum inhibitory concentration (MIC) effects (PA-SME), and sub-MIC effects (SME) of chlorhexidine on oral bacteria were investigated.

**Methods**

For PAE measurement, bacteria were exposed to 10x MIC chlorhexidine for 1 min, which was then eliminated by washing. For determining PA SME, bacteria were exposed to 0.1, 0.2, and 0.3x MIC chlorhexidine during the postantibiotic phase and to sub MIC chlorhexidine for measurement of SME. PAE, PA SME, and SME of chlorhexidine were observed.

**Conclusions**

The PAE lasted for 0.9 h for *Streptococcus mutans*, 0.1 h for *Streptococcus gordonii*, and 0.35 h for *Lactobacillus acidophilus*. The PA SME against oral bacteria lasted for longer duration as the chlorhexidine concentrations increased. The PA SME against oral bacteria lasted for substantially longer than SME did. The present study illustrates the existence of chlorhexidine-induced PAE, PA SME, and SME against oral bacteria, thereby extending the pharmacodynamic advantages of chlorhexidine.

**FEMS-0485**  
**Free subjects**

## **PINWORM INFECTION AMONG FOREIGN LABORERS IN TAIWAN**

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### **Background**

According to the demographic ageing of labor force participation in Taiwan, we imported massive foreign laborers to Taiwan for nursing or other labor force work since October, 1989. The purposes of this study was to evaluate the effectiveness of single examination to diagnose Enterobiasis by scotch tape perianal swab technique in non-constant time.

### **Objectives**

The survey covered southeast asian laborers from four countries including philippines, thailand, Indonesia and vietnam. The data were collected from 391 valid questionnaires, 391 valid stool samples and 391 valid scotch tape perianal swab samples.

### **Methods**

Single scotch tape perianal swab technique was performed by foreign laborers in non-constant time. Stool samples were also obtained after the scotch tape perianal swab. This was a cross-sectional study. Descriptive statistics were applied to the data.

### **Conclusions**

Scotch tape perianal swab technique in non-constant time could increase the detection rate of Enterobius vermicularis from 0% to 2.0%(8/391). Occupational health personnel should educate the foreign laborers to get into the habit of washing hands before a meal in their daily practice. The employers should arrange the examination of scotch-tape anal swab technique for caretakers periodically.



**FEMS-2544**  
**Free subjects**

## **SPECIFIC KILLING OF BACTERIA USING A TOXIN-INTEIN BASED AND-LOGIC GATE**

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### **Background**

Synthetic biology aims reprogram bacteria to design and construct sophisticated new biological systems by gene regulatory networks. However gene regulatory networks (gene expression, protein or molecule concentration) are continuous, thus fitting them into a deterministic model like Boolean approximation (0/1) is noisy.

### **Objectives**

We would like to implement a model aiming at killing specific bacteria in mixed populations. We will design an AND-logic gate to solve Boolean satisfiability (SAT) problem. Our model is based on conjugation where plasmids will act as wires (plasmid inside cell is 1; no plasmid is 0).

### **Methods**

To avoid the noise associated with transcriptional regulators, our system is based on half toxins (thus non-functional) coupled with split intein technology. We are designing two types of plasmids: input plasmids (A and B) and clause plasmid (CP). Only CP is conjugative. A population of bacteria could have already implemented the input plasmids in different combinations: A and B; only A; only B or no one. The output in our system is *cell death*. The CP will infect these bacteria by conjugation and it will evaluate the inputs and will specifically kill bacteria harboring both plasmids (A and B). When both plasmids are together in the same cell, the expression of the toxin-intein fusion triggers the reaction between two inteins domains, allowing the reconstitution of the toxin and killing the cell.

### **Conclusions**

We have successfully completed the evaluation of Boolean-SAT problem by the construction of an AND-logic gate and using the inputs plasmids A and B, and the CP.



**FEMS-2634**  
**Free subjects**

## **OUTBREAK OF BOTULISM IN MEMBERS OF A FAMILY FOLLOWING THE CONSUMPTION OF KUSHK**

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### **Background**

Botulism is a serious neuromuscular disease caused by the *Clostridium botulinum*

### **Objectives**

In this article, we report an outbreak of the foodborne botulism due to consumption of kushk in 5 members of a family

### **Methods**

case presentation

A 41 and 46 year old women presented with diplopia and blurred vision from 4 days ago. She had progressive symptoms including dry mouth, dysphagia and swallowing disorder, constipation, dyspnea, general weakness of neck muscle and lower and upper limbs. Pupils were dilated and nonreactive to light. There was no gag reflex. Deep tendon reflexes were normal and the muscle force of upper limb was 4/5.

The third case was a 13 year old boy presented some days after two above mentioned cases. His complaints were ptosis, diplopia, dry mouth, vertigo and weakness of lower and upper limbs but had no constipation, dysphagia and swallowing disorder and dyspnea. The fourth case was a 12 year old boy with history of diabetes and similar symptoms and signs and the fifth case was an 8 year old girl with neck rigidity and ptosis.

They were members of a family and had history of consumption of kushk. Interval between involvement of children was longer than adult and incubation period. Disease was more severe in adult than children. EMG was done in one of the women and was suggestive of botulism. After clinical diagnosis, serum of patients was obtained and the patients received antitoxin. Their symptoms resolved gradually.

### **Conclusions**

The diagnosis of botulism should be made based on the patient's initial signs and symptoms and history of consumption of suspected food.

**FEMS-2657**  
**Free subjects**

## **BLOOD BORN DISEASES IN INTRAVENOUS DRUG ABUSERS**

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### **Background**

Drug injection is responsible for numerous minor to life-threatening and fatal complications such as the transmission of human immunodeficiency virus (HIV), sexually transmitted diseases, and viral hepatitis

### **Objectives**

this paper has tried to offer a brief look at the epidemiology of drug abuse, while focusing on blood born infections seen in IDUs.

### **Methods**

The study was carried out using a cross sectional design. A questionnaire was distributed among 135 IDUs. serological tests (ELISA Anti HCV, Anti HIV, HBs Ag) were taken to confirm the existence of blood borne diseases

### **Conclusions**

A total of 135 cases between the ages of 15-57 were studied. opium was most frequently first drug used (58.5%), followed by cannabis (20.7%) and heroin (11.1%) .

nearly 68.9% of the population began abusing drugs before the age of 20. 61.4% of the cases had a history of sharing needles Other results showed that 51.5% had an accompanying blood borne disease. Further analysis indicated a meaningful relationship ( $P < 0.001$ ) between needle sharing and accompanying blood borne diseases. Of the 51.5% who had an accompanying blood borne disease, 17.1% were found to have either reactive ELISA of AntiHIV alone, or in combination with Hepatitis. In other words, it can be concluded that 8.1% of the total population had reactive ELISA of AntiHIV. Results also showed that 11.1% of the total population had HBs Ag positive while 47.4% had Positive ELISA Anti HCV

**FEMS-2662**  
**Free subjects**

## **MICROBIAL CAUSES OF WOUNDS AND SKIN INFECTIONS IN INJECTING DRUG USERS**

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### **Background**

60–80% of hospital admissions in IV drug abusers are due to infectious complications and skin and soft-tissue infections seem to be some of the most common infections among this group

### **Objectives**

The goal of this study was to identify the characteristics of wounds and skin infections of this group to hopefully have early recognition and as a result better prevention and management strategies

### **Methods**

This descriptive cross sectional study was carried out on 135 IDUs . . all current injection drug users who had at least one wound as a result of injecting drugs were enrolled in this study. the wounds were physically examined and smear samples were also cultivated on Blood Agar and EMB Agar environments for microbiological assessments

### **Conclusions**

A total of 135 cases between the ages of 15-57 were studied. T. Most wounds (45.2%) were located in the lower limbs such as the foot, . the majority (44%) of wounds were as old as six months up to one year. each individual had at least 4 wounds with an average size of 1.8 cm<sup>2</sup>. 22.2% of the population had some form of infection such as abscess or cellulites. Thrombophlebitis was seen in 37% and. Only 10.4% showed necrotizing ulcers .

Microbiological assessments found that the majority of wounds (54.1%), had gram positive cocci. This included staphylococcus (36.3%), streptococcus (17.8%). 7.4% of the cases showed gram negative species. 30.4% of the bacterial cultures showed negative or nontypable results, since the culture environments used were very routine and non-specific. Fungi were seen in 8.1% of the cases



**FEMS-2663**  
**Free subjects**

## **MICROBIAL CAUSES OF WOUNDS AND SKIN INFECTIONS IN INJECTING DRUG USERS**

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**FEMS-2668**  
**Free subjects**

## **OUTCOME OF HIV/AIDS PATIENTS WITH PNEUMONIA**

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### **Background**

Pneumonia is also a major cause of prolonged hospitalization, mortality and morbidity among HIV infected people that costs millions each year

### **Objectives**

the goal of this study was determine of outcome and prognostic factors of pneumonia in HIV positive individuals.

### **Methods**

In a descriptive method, 80 HIV positive patients that hospitalized in Loghman-hakim hospital between 2005 to 2011 with pneumonia have been studied and their demographic findings include age ,sex, marriage, job, social history such as smoking, Iv drug using and imprisonment, medical history of co infections such as pulmonary TB, HCV and HBV, and also clinical features include their initial signs and symptoms ,lab data and imaging, and at last their outcome gathered and record in questionnaire. Then gathered data enter SPSS#13 software, analyzed by descriptive tests

### **Conclusions**

80 HIV positive patients hospitalized with pneumonia in loghman-hakim hospital since 2005 to 2011 entered the study. Mean age was 37.4(±8.2) years. death was the first with 43.8% , then recovery with 30% and pulmonary complications with 16.3% were the most common outcomes. Mortality was more common in the elderly .Most of the patients had history of smoking,injection drug use and. imprisonment history and mortality rate in these patients were more common. The mortality rate was also more common in patients with anemia, high ESR levels, CRP + , high urea and high creatinine levels and low CD<sub>4</sub> counts and pleural effusion.

**FEMS-2300**  
**Free subjects**

## **SCREENING OF SIDEROPHORE BIOSYNTHESIS GENES FROM SIDEROPHORE PRODUCING ACTINOMYCETES**

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<sup>1</sup>*Biology, Chiang Mai University Faculty of Science, Chiang Mai, Thailand*

### **Background**

Iron is an essential trace element required for growth of all microorganisms. However, iron in environment is mostly insoluble form and cannot be readily utilized by microorganisms. In iron starvation condition, actinomycetes have developed specific mechanisms involving in the production of small organic metal chelator called siderophore.

### **Objectives**

Actinomycetes were isolated from coastal and mangrove sediments in Thailand. Genomic DNA were extracted by phenol-chloroform method. Hydroxamates and catecholate siderophore biosynthetic genes were detected by PCR using *desD* and *entF* specific primers, respectively. Siderophore production was determined by CAS assay. Siderophore producing actinomycetes were identified by 16S rRNA gene sequence analysis.

### **Methods**

A 1.2 kb fragment of *desD* gene that encodes for desferrioxamine, a hydroxamate siderophore was detected in 7 isolates. A 203 bp amplified fragment of *entF* gene encoding enterobactin, a catecholate siderophore was detected in 3 isolates. All isolates produced siderophore on Chrome Azurol S agar. High siderophore producing *Streptomyces parvulus* S2-SC3 was selected for sequence analysis of *desD* gene. A 1.2 kb amplified PCR fragment showed 90% sequence similarity with desferrioxamine synthetase from *Streptomyces lividans* strain 1326. Phylogenetic analysis revealed that these siderophore producing actinomycetes were members of the genus *Micromonospora*, *Streptomyces* and *Verrucosispora*.

### **Conclusions**

PCR offers a fast way to screen for siderophore producing actinomycetes. However, some producing strains were failed to detect by our PCR screening, several actinomycetes strains may carry diverse siderophore biosynthesis gene which could not be detected by currently available primers. Our results suggested that novel siderophore may exist in these actinomycetes.



**FEMS-2301**  
**Free subjects**

## **TAXONOMIC DIVERSITY OF ACTINOMYCETES FROM THAI MANGROVE SEDIMENTS**

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<sup>2</sup>*Marine Science, Burapha University, Chonburi, Thailand*

<sup>3</sup>*Biology, Chiang Mai University Faculty of Science, Chiang Mai, Thailand*

### **Background**

Actinomycetes are large group of high GC Gram positive bacteria that widely distributed in nature. They have proved to be an important source for industrially useful bioactive metabolites, notably antibiotics. In Thailand, there are very few studies on microbial diversity in mangrove forests. In this study, we report on the diversity of cultivable actinomycetes in mangrove sediments and provide taxonomic evidence of their novelty taxa.

### **Objectives**

Actinomycetes were isolated from mangrove sediments and taxonomically characterized. Representative isolates were selected and identified based on 16S rRNA gene sequence analysis. Putative novel taxa were subjected to polyphasic taxonomic characterization using standard methods.

### **Methods**

Over 100 strains of actinomycetes were isolated from mangrove sediments using media selective for actinomycetes. Phylogenetic analyses based on a 16S rRNA gene sequences showed that the majority of isolates were dominated by genus *Streptomyces*. In addition, members of the genera *Amycolatopsis*, *Jiangella*, *Micromonospora*, *Nocardia* and *Verrucosipora* were also found. Some isolates may represent a new species. For example, *Jiangella* strain 3SM4–07<sup>T</sup> was most closely related to *Jiangella alkaliphila* JCM 15620<sup>T</sup> (99.0%), However, they shared mean DNA–DNA hybridization values of only 31.5±2.6%.

### **Conclusions**

Our results provide further evidence that mangrove sediments harbor taxonomically diverse actinomycetes. The diversity of these organisms and their novelty support the view that this under studied habitat is a rich source of novel actinomycetes which can be further exploited for biotechnological purposes.



**FEMS-2564**  
**Free subjects**

## **ACTINOMYCETES FROM EUCALYPTUS AND THEIR ACTIVITY TO INHIBIT EUCALYPTUS LEAF AND SHOOT BLIGHT DISEASE**

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### **Background**

Eucalyptus are the most important raw material supply for pulp industries in Thailand. Leaf and shoot blight caused by *Cryptosporiopsis eucalypti*, *Cylindrocladium* sp. and *Teratosphaeria destructans* is a serious disease in eucalypt plantations. The search of actinomycetes for biological control agents is one of the promising approaches.

### **Objectives**

In this study, we isolated actinomycetes capable of inhibiting the growth of fungal pathogens causing leaf and shoot blight on eucalyptus. The potential strain to use as biocontrol agent was selected for further study.

### **Methods**

Actinomycetes were isolated from roots and rhizospheric soil of eucalyptus using starch casein agar and humic acid-vitamin agar supplemented with antimicrobial antibiotics. The isolates were screened for their ability to inhibit eucalyptus fungal pathogens using dual culture technique.

### **Conclusions**

A total of 478 actinomycete strains were successfully isolated from roots (95 isolates) and rhizosphere soil (383 isolates) of eucalyptus. Among these, 439 isolates (91.8%) were streptomycetes and 39 isolates (8.16%) were non-streptomycetes. All isolates were evaluated for their *in vitro* antagonistic effect on plant pathogenic fungi. The result showed 273, 120 and 241 isolates were antagonistic to *Cryptosporiopsis eucalypti*, *Cylindrocladium* sp. and *Teratosphaeria destructans*, respectively. Among these, 48 isolates (10%) displayed activity against all three fungal pathogens. The isolate EUSKR2S82 showed the strongest inhibitory effects against all tested fungi.

The identification of this strain using 16S rRNA genes sequence revealed that isolate EUSKR2S82 shared 99.4% similarity with *Streptomyces ramulosus* NRRL B 27-14<sup>T</sup>.



**FEMS-2276**  
**Free subjects**

## **CULTIVABLE ACTINOMYCETES ASSOCIATED WITH GLOMUS MOSSEAE SPORES AND THEIR PLANT GROWTH PROMOTING ACTIVITY**

*K. Lasudee<sup>1</sup>, W. Pathom-aree<sup>1</sup>*

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### **Background**

Actinomycetes are prolific producers of bioactive compounds. Much of our research effort has been directed at assessing the diversity of actinomycetes in various environments. Currently, we are interested in mycorrhizal helper bacteria which may be a good source for novel taxa for bioprospecting.

### **Objectives**

In this study, we report on the isolation of actinomycetes from spores of *Glomus mosseae* and provide preliminary evidence of their potential in agriculture as plant growth promoter.

### **Methods**

Actinomycetes were isolated from spores of *Glomus mosseae* using the dilution plate technique and media designed for the selective isolation of members of specific actinomycete taxa. All isolates were identified by 16S rRNA gene analyses. The isolates were examined for their ability to produce indole-3-acetic acid, siderophores and solubilize phosphate *in vitro*.

### **Conclusions**

Six strains were isolated from spores of *Glomus mosseae* using media selective for actinomycetes. Phylogenetic analyses based on a 16S rRNA gene sequences showed that the isolates belonged to the genera *Lysibacter* and *Streptomyces*. All isolate produced siderophore, four isolates produced IAA and two isolates solubilized phosphate at varying level. *S. thermocarboxydus* S3 produced 11.23±0.02 mg/ml IAA and high activity of phosphate solubilization and siderophore production. The results provide evidence that actinomycetes were associated with arbuscular mycorrhizal spores of *Glomus mosseae*. The ability of these organisms to produce plant growth promoting agents support the possibility of using these actinomycetes for agricultural purposes.

**FEMS-2591**  
**Free subjects**

**PROFERROROSAMINE A SECRETION BY ERWINIA RHAPONTICI P45 INHIBITS GROWTH OF THE FIRE BLIGHT PATHOGEN ERWINIA AMYLOVORA**

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<sup>2</sup>Zurich University of Applied Sciences, Institute of Food and Beverage Innovation, Wädenswil, Switzerland

**Background**

The classic diagnosis of the fire blight pathogen *E. amylovora* (EA) takes the cell morphology on King's B agar into account. Sometimes, an unusual pink coloration of EA colonies can be observed on this agar. This coloration is due to the co-cultivation of *E. rhapontici* or *E. persicina* and their production of the iron (Fe<sup>2+</sup>) chelator proferrosamine A (pFRA), that accumulates in *E. amylovora*. Dependent on the proximity of a pFRA synthesizer, EA colonies can also exhibit an attenuated growth.

**Objectives**

To get a deeper insight into the mechanism of the Fe<sup>2+</sup> chelator, we aimed to identify the genes involved in proferrosamine A synthesis.

**Methods**

pFRA negative mutants of *E. rhapontici* P45 were isolated in a phenotypic transposon mutagenesis screen.

**Conclusions**

We identified a 9.3 kb cluster of seven genes that encode proteins involved in pFRA synthesis. Wild type *E. rhapontici* P45, but not pFRA negative mutants, showed a growth inhibition effect on EA *in vitro* and detached flower assay.

**FEMS-1829**  
**Free subjects**

**COMBINED EFFECT OF OSMOTIC AND UV-B IRRADIATION ON A N<sub>2</sub> FIXING  
CYANOBACTERIUM FROM A RICE PADDY**

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**Background**

Cyanobacteria play a crucial role in the productivity of terrestrial and aquatic systems and have developed a series of defense mechanisms against abiotic factors as UV-B light and osmotic stress.

**Objectives**

In order to study the protective strategies of *Calothrix* B122 spp., this cyanobacterial isolate from a subtropical rice field was exposed to low doses of UV-B alone or in combination with osmotic stress.

**Methods**

After the stress exposure, oxidative damage was determined by TBARS and proline accumulation quantified. The activity of enzymes (superoxide dismutase and catalase) involved in the decrease of reactive oxygen species (ROS), O<sub>2</sub><sup>-</sup> or H<sub>2</sub>O<sub>2</sub>, were determined and the *in vivo* production of ROS was followed by confocal microscopy. Photosystem II yield and O<sub>2</sub> photoevolution were also studied as proxies of the effect of the stress on the cyanobacterium physiology.

**Conclusions**

Osmotic stress, in general, caused a more pronounced oxidative damage but in combination with UV-B the damage drastically increased. Among the enzymes involved in the antioxidant defense, CAT had a higher activity under osmotic stress but in the presence of both types of stresses its activity doubled. SOD, on the other hand, had higher rates only under UV-B light. The accumulation of ROS followed a similar pattern as the antioxidant enzymes studied. Photosynthesis was affected in all of the situations studied. This study showed that this cyanobacterium had a differential response when exposed alone to each type of stress than in combination.

**FEMS-2774**  
**Free subjects**

## **TAILORING AGAROSE WITH PENDANT GROUPS FOR DISTINCT DNA FRAGMENT ANALYSIS IN GEL ELECTROPHORESIS**

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### **Background**

Agarose gel electrophoresis has been widely used as the standard method to separate, identify, and purify DNA/RNA fragments in the life sciences research, because it's easy to perform, relatively inexpensive, and excellent in analytical performance characteristics. However, technologies to separate for short DNA fragments in high resolution are still lacking.

### **Objectives**

In response to the above challenge, we hypothesized that the tailoring agarose molecules with various pendant groups would provide the rapid analysis of an array of small to large DNA fragments in the gel electrophoresis.

### **Methods**

We examined this hypothesis by tailoring the agarose molecules with various pendant groups such as octadecyl chains, and aromatic groups, and then directly fabricate the three dimensional agarose gel for electrophoresis. This concept of the hydrophobically-modified 3D microporous gel can be regarded also as a novel microfluidic-based mold for being demonstrated and also used in the bio-microelectromechanical system (Bio-MEMS). The molecular structure (degree of substitution and molecular weight) of the modified agarose was confirmed by <sup>1</sup>H-NMR, and MALDI-TOF (Matrix: CHCA) analysis. Interestingly, the tailoring agarose gel provided the distinctive separation of small DNA fragments efficiently and also enhanced the discriminatory power in the gel electrophoresis based on the pendant groups' characteristics.

### **Conclusions**

Overall, the results from this study may be useful in understanding the role of the hydrophobic/hydrophilic balance in the separation of DNA fragments and further in the extracellular microenvironments in tuning a variety of cellular activities.

**FEMS-0774**  
**Free subjects**

**TEMPERATURE- AND NITROGEN SOURCE-DEPENDENT GROWTH OF  
LISTERIA MONOCYTOGENES IMPLICATES AN IMPORTANT ROLE OF 2-  
OXOGLUTARATE AS INTERNAL SIGNAL DURING ADAPTATION TO CHANGING  
ENVIRONMENTS**

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**Background**

The pathogen *Listeria monocytogenes* lives either saprophytically in the environment or within cells in the vertebrate host. Thus it has to adapt its lifestyle to its ecological niche and the availability of nutrients.

**Objectives**

The aim of this study was to investigate the impact of different nitrogen sources and temperatures on growth of *L. monocytogenes*.

**Methods**

Nitrogen source dependent growth analysis was conducted in defined minimal medium at 24°C or 37°C with either glutamine or ammonium as nitrogen source. Nitrogen source- and temperature-dependent gene transcription was validated by qPCR.

**Conclusions**

A preference for ammonium over glutamine was observed in growth experiments. This might be ascribed to the interrupted TCA cycle in *L. monocytogenes*, causing accumulation of the intermediate 2-oxoglutarate. 2-oxoglutarate has to be removed to allow a permanent glucose metabolism by continuous channeling of pyruvate in the TCA cycle. During growth on ammonium twice the amount of 2-oxoglutarate is removed compared to growth on glutamine.

Reduced growth on glutamine was more obvious at 24 °C than at 37 °C. When grown on the same nitrogen source, but at different temperatures, the removal of 2-oxoglutarate is mainly determined by the amount of glutamate used for the *de novo* biosynthesis of amino acids which is expected to be elevated at the optimal growth temperature of 37 °C.

It is assumed that in *L. monocytogenes* the intracellular 2-oxoglutarate concentration serves as internal signal that allows the adaptation of *L. monocytogenes* to two environmental parameters, temperature and nitrogen source, at the same time.

**FEMS-1103**  
**Free subjects**

## **INVESTIGATION OF AUTOANTIBODIES IN CANCER PATIENTS**

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### **Background**

Malignant diseases are often related with the induction of autoimmunity, characterized by the generation of autoantibodies against a wide range of autoantigens. Serum autoantibodies have been identified in patients with solid tumors and in patients with hematological malignancies.

### **Objectives**

The purpose of the study was to investigate the prevalence of antinuclear antibodies (ANA) in sera of the patients with various cancer types.

### **Methods**

Serum samples were obtained from 54 patients with cancer (37 breast, 1 *myxoid liposarcoma*, 1 brain, 2 lung, 1 colon, 3 gastric, 1 pancreas, 2 lymphoma, 1 multiple myeloma, 1 nasopharynx, 1 renal, 1 prostate, 1 vulva, 1 endometrial) and with rheumatic complaint. A total of 100 healthy blood donors served as the study control group. ANA patterns were searched by using the HEp-2010/Liver (Monkey) indirect immunofluorescence assay (IFA) kit (Euroimmun AG, Germany). In addition these samples were further processed by line immunoassay (LIA), (Euroimmun AG, Germany).

### **Conclusions**

Of 54 patients, 18 were ANA-positive by IFA and LIA. Homogen (8), granular/speckled (2), nucleolar (2), anticontromere (2) and mixed (4) patterns were detected by IFA. SS-A/Ro (8), SS-B/La (6), antidsDNA (2), antiSci (2) and CENP-B (2) positivity were observed by LIA. In healthy control group, ANA was not detected. There was statistically significant difference between the results of the patients and of the control group. Of 54 patients, 29 were diagnosed with cancer while 20 were diagnosed with rheumatic disease firstly; and 5 were diagnosed with both simultaneously. In

conclusion, the association between rheumatic diseases and malignant diseases need further investigation.

**FEMS-1151**  
**Free subjects**

## **ANTI-DFS70 ANTIBODIES IN 500 HEALTHY BLOOD DONORS**

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### **Background**

Autoantibodies against DFS70 (dense fine speckled 70) antigen have been recently identified among the antinuclear antibodies (ANAs) in patients with systemic autoimmune rheumatic diseases.

### **Objectives**

The typical DFS has been described as indirect immunofluorescence (IIF) staining pattern which is uniformly distributed throughout the interphase nucleus and, most notably, are localized on metaphase chromosomes. We undertook this study to examine the frequency of anti-DFS70 antibodies in a large number of healthy blood donors and to evaluate the significance of these antibodies, accordingly.

### **Methods**

Sera of 500 healthy blood donors (459 men, 41 women) were analyzed for ANAs and anti-DFS70 antibodies by IIF with HEP-2 cells as a substrate.

### **Conclusions**

ANAs were determined in 0.8% of all blood donors by IIF. Positive results were detected in different serum dilutions: Three subjects (0.6%) 1:320 nucleolar pattern, and 1 subject (0.2%) 1:1000 homogeneous/nucleolar pattern. There were 3 anti-DFS70 antibody positive blood donors. In this study, anti-DFS70 autoantibody was detected 0.6% in healthy blood donors. Among anti-DFS70 antibody-positive subjects the percentage of male was 0.65% (3 of 459 subjects). The prevalence of anti-DFS70 antibody positivity decreased with increasing age. According to our results, anti-DFS70 autoantibody is detected more commonly in male than female healthy blood donors. More comprehensive studies are needed to be done to investigate the anti-DFS70 autoantibody specificity for healthy individuals.



**FEMS-1166**  
**Free subjects**

## **ANTI-DFS70 ANTIBODIES IN SYSTEMIC AUTOIMMUNE RHEUMATIC DISEASES**

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### **Background**

Systemic autoimmune rheumatic diseases are major cause of morbidity and mortality. The presence of various autoantibodies, such as antinuclear antibodies produced against intracellular antigens, are characteristic of systemic autoimmune rheumatic disease (SARD).

### **Objectives**

In this study, we aimed to investigate the prevalence of anti- DFS70 antibodies in SARD and to evaluate the significance of these antibodies, accordingly.

### **Methods**

In our study we created test groups as; 107 healthy blood donors, 418 Rheumatoid arthritis (RA), 101 Systemic Lupus Erythamatosus (SLE) patients, 36 Systemic sclerosis patients, 71 Sjögren syndrome patients, 43 Ankylosing spondylitis and 2555 Connective tissue disease (CTD) pre-diagnosed patients. Serum samples were evaluated by indirect immunofluorescence assay (IFA). Anti-DFS70 positive samples were also tested by ELISA test.

### **Conclusions**

Anti DFS70 antibody was detected 1.4% in RA, 2.97% in SLE, 1.4% in Sjögren syndrom and 1.05% in disease group with pre-diagnosed CTD. It was not detected in AS and SSc patient groups. Totally 39 out of 47 samples were also found to be anti DFS70-positive by ELISA method. According to our results, anti-DFS70 autoantibody is detected more commonly in certain disease sub-groups of SARD than healthy individuals.

**FEMS-0738**  
**Free subjects**

## **NONTOXIGENIC CLOSTRIDIUM DIFFICILE MEMBRANE FRACTION AS A VACCINE CANDIDATE**

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### **Background**

*Clostridium difficile* is a leading cause of infectious diarrhea and pseudomembranous colitis. Standard treatment for *C. difficile* infections (CDI) includes the administration of antibiotics. However, the rate of CDI recurrence after initial therapy is approximately 20% and even increases when antibiotic therapy is repeated.

### **Objectives**

Therefore, alternatives or additional measures to current antibiotic therapy are required to achieve lower rates of CDI recurrence. Vaccination is one of prevention measures by which low recurrence rate is expected. In this study, we demonstrate the potential of nontoxigenic *C. difficile* membrane fraction (ntCDMF) as a vaccine candidate.

### **Methods**

Mice immunized with ntCDMF showed serum IgG and intestinal IgA titers of 4,660 and 156, respectively, as compared to 30 and 13, respectively, for non-immunized controls. In Caco-2 cell adhesion assay for 9 different *C. difficile* strains, the number of adherent bacterial cells pretreated with the immune serum was 5.8–9.3 CFU per 100 Caco-2 cells, which was significantly lower than in the case treated with non-immunized serum (21.1 to 36.6). Similar findings were observed for intestinal fluid, where the number of adherent bacterial cells ranged from 19.6 - 29.1 and 25.9 - 42.0 CFU per 100 Caco-2 cells for immunized and control mice, respectively.

### **Conclusions**

These results support the idea of ntCDMF-derived *C. difficile* vaccine capable of preventing the adhesion for *C. difficile* to the intestinal cells, as well as emphasize its potential as a preventive measure against CDI.

**FEMS-1997**  
**Free subjects**

## **TOXICITY AND ANTIBACTERIAL ACTIVITY OF CYANOBACTERIAL STRAINS ISOLATED FROM SERBIAN FRESHWATERS**

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### **Background**

This paper reviews the toxicity and antibacterial activity of ten cyanobacterial strains isolated from fresh waters in Serbia.

### **Objectives**

All investigated cyanobacterial strains showed toxic effect to *A. salina*. The highest toxicity was observed in the case of strains belonging to *Microcystis* genera (*Microcystis* L1 and P1) with detected larval mortality of 98% and 96%, respectively. The observed LD50 values were from 0.24 mgml<sup>-1</sup> (*Anabaena* P1) to 3.37 mgml<sup>-1</sup> (*Oscillatoria* K3). The intracellular microcystin concentrations, calculated as microcystin-LR equivalents, were in the range of 0.1–3.96 µgmg<sup>-1</sup> dry weight. All cyanobacterial strains revealed antibacterial activity and inhibited the growth of at least one bacterial species. The intracellular extract of *Aphanizomenon* K2 strain showed the most evident inhibitory effects to 6 bacteria. The MIC values of the tested strains were in the range of 0.00014 gL<sup>-1</sup> - 0.5805 gL<sup>-1</sup>.

### **Methods**

The toxicity of intracellular extracts of cyanobacterial strains was tested by *Artemia salina* bioassay and expressed as the percentage of larval mortality. The intracellular hepatotoxin (microcystin) content of cyanobacterial strains was determined by colorimetric protein phosphatase 1 inhibition assay (PP1). Methanolic intracellular extracts of cyanobacterial strains were tested for antibacterial activity using agar diffusion method and broth microdilution assay.

### **Conclusions**

The results suggest that toxic effect on larvae *A. salina* found in the most tested strains is likely to be a consequence of their microcystin production. The obtained results indicate that toxins are probably not responsible for the expressed antibacterial activity of the tested strains.

**FEMS-0919**  
**Free subjects**

## **LABELLING OF EICOSAPENTAENOIC ACID WITH STABLE ISOTOPE ( $^{13}\text{C}$ ) IN BACTERIA**

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### **Background**

Eicosapentaenoic acid (EPA) is an essential omega-3 polyunsaturated fatty acid (PUFA) well known for its beneficial effects on human and animal health.

EPA is present at relatively high levels in a number of marine animals with its primary dietary source being microalgae and some bacteria. This essential bioactive plays a critical role in marine life; however its transfer between different trophic levels is yet to be fully understood.

### **Objectives**

A simple and affordable method to study and trace the fate of EPA through the trophic food chain was developed.

### **Methods**

*Shewanella* sp. is a marine bacterium, known to produce EPA [1, 2]. In this work, we describe a simple method to label the EPA present in the bacterial strain IRL 567 (*Shewanella* sp.) with the stable isotope  $^{13}\text{C}$ . Labelling assays were performed at small scale (100 ml shake flask) and bench scale (1lt stirred tank bioreactor). By incubating the bacteria with  $^{13}\text{C}$ -acetate in culture medium, we demonstrated that EPA is *de-novo* synthesized utilizing the simplest carbon source -acetate- as precursor.  $^{13}\text{C}$  incorporation into the EPA molecule was determined by mass spectrometry (ESI TOF MS), finding that 95.5% of synthesized EPA being labelled in the shake flask and 88.6% in the bioreactor, yielding a concentration of labelled EPA of 5.36 mg/l and 2.90 mg/l, respectively, with between 2 to 8  $^{13}\text{C}$  atoms contained in EPA structure.

### **Conclusions**

Labelled EPA could be a valuable research tool to understand the assimilation and metabolic routes of fatty acids, essential components in human and animal diets.



**FEMS-2011**  
**Free subjects**

**BLOODSTREAM FUNGAL ISOLATES DURING 8-YEAR PERIOD: 2007-2014**

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**Background**

The occurrence of candidemia is on a rise worldwide. Non-albicans *Candida* species have emerged as major causes of candidemia in many countries. Added to it is the problem of antifungal resistance in *Candida* isolates.

**Objectives**

The aim of our study was to investigate the isolation of *Candida* spp in blood cultures and to evaluate their antifungal susceptibility during a 8-year period (2007-2014) in a tertiary care hospital.

**Methods**

The blood cultures were incubated in the automated blood culture system BACTEC9240 (Becton Dickinson). Positive blood cultures were examined microscopically directly for yeast or pseudohyphae and subcultured on Sabouraud dextrose agar (Liofilchem Italy). *Candida* isolates were identified using automated VITEK 2 system (bioMérieux) or Api 20CAUX (bioMérieux). Antifungal susceptibility was carried out by automated VITEK 2 system (bioMérieux) using AST Y01 or AST Y07 test card.

**Results:** During the study period there were 67 candidemia cases. The 51.35% of candidemias occurred in the ICUs, the 40.54% in the medical wards and the rest of 8.11% in surgical wards. *C. parapsilosis* was the predominant species (35.15%), followed by *C. albicans* (32.43%), *C. glabrata* (10.81%), *C. tropicalis* (8.12%) and *C. norvegensis*, *C. guilliermondii*, *C. kefyr*, *C. famata*, *Cryptococcus neoformans* with (2.70%). All tested isolates were susceptible to amphotericin B and voriconazole. Among non-albicans strains increasing resistance to fluconazole was found: *C. parapsilosis* (8.33%), and *C. tropicalis* (8.33%).

**Conclusions**

Candidemia was more frequent in ICUs followed by medical and surgical wards. *C. parapsilosis* was the predominant cause of candidemias in our Hospital. Amphotericin B and voriconazole were active against all tested isolates.



**FEMS-0995**  
**Free subjects**

**ASSESSMENT OF IMMUNE PROTECTIVE CAPACITY OF THE RECOMBINANT IRON SUPEROXIDE DISMUTASE (FESOD) FROM BORDETELLA PERTUSSIS**

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**Background**

Whooping cough (pertussis) is a highly contagious respiratory infection caused by *Bordetella pertussis*. Although availability of effective pertussis vaccines seems to decrease the incidence of the disease, *B. pertussis* circulation in population has not been eliminated. Thus, finding new protein candidates with high immune protective capacities is necessary to enhance the efficacy of current acellular pertussis (Pa) vaccines (1).

**Objectives**

The aim is to evaluate immune protective capacity of iron superoxide dismutase (FeSOD) protein from *B. pertussis* with the aim of developing new generation pertussis vaccines.

**Methods**

FeSOD gene was cloned, expressed in *Escherichia coli* and the recombinant protein was purified. FeSOD was formulated with Aluminum hydroxide (Alum) or Monophosphoryl Lipid A (MPLA) and mice were immunized intraperitoneally. IgG1, IgG2a and IFN- $\gamma$  levels were determined and bacterial colonization on mice lungs were evaluated.

**Conclusions**

IgG1 and IgG2a responses were significantly increased in both mice groups immunized with FeSOD-Alum and FeSOD-MPLA, the level of IgG2a was relatively higher in mice vaccinated with FeSOD-MPLA. Immunization with FeSOD-MPLA formulation provided a significant decrease in bacterial count in mice. Moreover, antigen specific-IFN- $\gamma$  response was significantly increased in the group vaccinated with FeSOD-MPLA. These findings, altogether, suggested that the recombinant FeSOD protein formulated with MPLA can be a possible acellular pertussis vaccine candidate.

**References:**

1. Heikkinen, E., Kallonen, T., Saarinen, L., Sara, R., King, A.J., Mooi, F.R., Soini, J.T., Mertsola, J., He, Q., (2007). PloS ONE 2(9): e904.



**FEMS-2934**  
**Free subjects**

**MIRRI CLUSTERS OF EXPERTISE**

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**Background**

MIRRI (Microbial Resources Research Infrastructure, [www.mirri.org](http://www.mirri.org)) is an initiative within the European Strategy Forum on Research Infrastructure (ESFRI) that includes 16 partners (14 public microbial Culture Collections / mBRCs), supported by 21 collaborators; the preparatory phase is currently funded by the European Commission.

MIRRI aims to construct a pan-European distributed infrastructure that boosts research and development in the field of biotechnology by improving access to the microbial resources present in European public collections, their associated data and expertise.

**Objectives**

One of the MIRRI objectives is to create clusters bringing together the expertise available at different partner and non-partner institutions to respond to concrete demands of the MIRRI stakeholders. These expert clusters will provide solutions in aspects such as regulatory framework (e.g. biosecurity, ABS, IPR), managerial best practices, taxonomy or microbial applications, among others.

**Methods**

To design the content, composition and rules of operation of the clusters, the relevant stakeholders have been consulted (face to face meetings, workshops and surveys). An analysis of the comparable RIs of the ESFRI 'Food and Health' group was performed to bridge the gap between information sharing and facilitating a collaboration landscape.

**Conclusions**

Here, we present the concept of the MIRRI Clusters of expertise as a tool to share and generate knowledge within the MIRRI stakeholder community (culture

collections, policy makers, scientists, bioindustry, etc.) to foster innovation within science, research and development. The authors gratefully acknowledge the contribution of the MIRRI consortium and the consulted persons to this work.

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**FEMS-1955**

**Bacterial nanomachines**

**GROWTH RATE CONTROL OF FLAGELLAR ABUNDANCE DIFFERS IN  
ESCHERICHIA COLI AND SALMONELLA ENTERICA SV. TYPHIMURIUM**

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**Background**

The bacterial flagellum is a complex organelle requiring the coupling of gene expression to the assembly pathway. In *Escherichia coli* and *Salmonella enterica* serovar Typhimurium the *flhDC* operon encodes the essential FlhD<sub>4</sub>C<sub>2</sub> flagellar-specific master transcriptional regulator. The activity of FlhD<sub>4</sub>C<sub>2</sub> is influenced by many flagellar-specific and global regulatory stimuli. FlhD<sub>4</sub>C<sub>2</sub> activity responds to cellular and environmental factors through regulators including OmpR, RcsB and CRP. Flagellar-specific signals known to influence FlhD<sub>4</sub>C<sub>2</sub> activity are controlled by the action of FlhT and FlhZ. *flhDC* expression is also sensitive to cell growth.

**Objectives**

Our objective in this study was to investigate the impact of growth rate on flagellar formation in *E. coli* and *S. Typhimurium*

**Methods**

Our methods to study our objective included the use of steady-state chemostat cultures. Our measure was flagellar abundance using a functional FlhM-GFP fusion protein. We will show that flagellar abundance correlates with growth rate, where faster growing cells produce more flagella in both species. We will present data showing that the impact of transcriptional, post-transcriptional and flagellar-specific regulation of *flhDC* with respect to the growth rate response of the flagellar system leads to a marked difference in flagellar abundance when the species are compared.

**Conclusions**

Our data suggests that even though both flagellar systems have a high degree of genetic similarity the way *E. coli* and *S. Typhimurium* assimilate signals during flagellar regulation may reflect the lifestyle of these two bacterial species.

**FEMS-1761**

**Bacterial nanomachines**

**TYPE VI SECRETION IN THE SOIL BACTERIUM PSEUDOMONAS PUTIDA:  
ROLE IN BACTERIAL COMPETITION**

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**Background**

Bacterial type VI secretion systems (T6SSs) are recently discovered nanomachines used to inject effectors into prokaryotic or eukaryotic cells. T6SSs are therefore involved in both inter-bacterial competition and bacterial pathogenesis.

**Objectives**

The aim of this work is the analysis of T6SS in the soil bacterium *Pseudomonas putida*. *P. putida* is known for its capacity to colonise the root of crop plants providing growth advantages to the plant and, importantly, protection against plant pathogens. Since T6SS is mainly used by environmental bacteria for interbacterial competition, we analysed whether this secretion system of *P. putida* might be involved in such protection.

**Methods**

- *In silico* analysis of *P. putida* KT2440 genome to identify potential T6SSs
- Competition assays to determine *P. putida* T6SS activity and bacterial targets.
- qRT-PCR and transcriptional fusions to study *P. putida* T6SS expression and regulation

**Conclusions**

*P. putida* contains three putative T6SSs named as H1, H2, and H3. These clusters contain the genes encoding the T6SS conserved core components and some accessory proteins, including regulatory proteins and toxins-immunity pairs. Additional T6SS-related genes are found scattered on the chromosome. At least the H1-T6SS is active and used by *P. putida* to kill other bacteria, including the phytopathogen *Pseudomonas syringae*. Expression of this system is maximal in stationary phase and it is controlled by the global regulators RetS and GacS-GacA, and by two alternative sigma factors, RpoS and RpoN.



**FEMS-1255**

**Bacterial nanomachines**

## **A PSEUDOMONAS FLUORESCENS TYPE VI SECRETION SYSTEM IS INVOLVED IN ANTIBACTERIAL ACTIVITY**

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### **Background**

To persist in an ecological niche, bacteria have developed several strategies to enable them to resist in the environment. One of these mechanisms able to struggle with other bacterial species is Type VI Secretion System (T6SS). This allows the bacterium to inject toxins directly into prey' cytoplasm and could cause death. A mucoid environmental strain of *Pseudomonas fluorescens*, MFE01, has antibacterial activity carried by T6SS. MFE01 is not virulent against eukaryotic cell models (amoebas, plant or animal cell models). Currently, genomic analysis reveals only one T6SS core component cluster and three *hcp* genes were found (named *hcp1*, *hcp2* and *hcp3*).

### **Objectives**

Aims of this study are to assign a role to each Hcp protein and to test the potential application of this secretion system to prevent infection by nosocomial strain (like *Pseudomonas aeruginosa*) in burn wound.

### **Methods**

Mutations of *hcp1*, *hcp2* and *hcp3* were performed. Furthermore, T6SS apparatus was also inactivated by *tssE* gene mutagenesis. Competitor activity were established in different conditions: immobilized experiment, swarming or swimming conditions. Hereafter, we will use Hacat keratinocyte model to study the ability of MFE01 to protect against infection by etiologic agents.

### **Conclusions**

MFE01 T6SS plays a crucial role in bacterial competition in ecological niche. Hcp2 was involved in antibacterial activity whereas Hcp1 immobilized prey cell. Hcp1 and Hcp2 proteins had a synergic effect which Hcp1 inhibits prey cell mobility and then Hcp2 can killed it. Presently, no role is attributed to Hcp3.

**FEMS-1363**

**Bacterial nanomachines**

## **MICRO- AND MACRO-SCALE INSIGHTS ON THE TRANSFORMATION OF SELENITE TO SELENIUM NANOPARTICLES BY MIXED MICROBIAL AGGREGATES**

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### **Background**

Selenium oxyanions can be reduced by diverse microorganisms resulting in the formation of selenium nanoparticles (SeNP). This process has been mostly investigated using pure bacterial cultures. However, using pure cultures for treatment of wastewater containing selenium oxyanions is not recommended. Anaerobic granular sludge, which consists of aggregates of mixed microbial communities, may also reduce selenium oxyanions and would thus be appropriate for developing treatment systems.

### **Objectives**

The aims were: (i) to investigate which microorganisms prevailed in a granular sludge that was exposed to selenite and reduced this oxyanion to SeNP, (ii) to investigate at the macro- and micro-scale the location of the produced SeNP, and (iii) to identify proteins associated to the SeNP.

### **Methods**

High-throughput pyrosequencing, electron microscopy and proteomic approaches were employed.

### **Conclusions**

A large change in the microbial community occurred when the inoculum granular sludge was exposed to and reduced selenite during a 20 day period. Most abundant microorganisms in the selenite reducing granular sludge were affiliated to *Veillonellaceae* and *Pseudomonadaceae* families. Most of selenium bioreduction occurred in the outer layer of the granular sludge, where most biomass contents are localized. At the micro-scale, electron tomography showed that SeNP were produced inside the cells. A proteomic analysis of extracted SeNP revealed that most of their associated proteins were proteins found in the outer and inner membranes. The used granular sludge readily reduced selenite and the combined results allowed proposing mechanisms for the bacterial synthesis of SeNP in these mixed microbial aggregates.

**FEMS-3084**

**Bacterial nanomachines**

**YERSINIA PSEUDOTUBERCULOSIS TYPE III SECRETION IS RELIANT UPON AN AUTHENTIC DUAL FUNCTIONAL N-TERMINAL YSCX SECRETOR DOMAIN.**

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**Background**

Various Gram negative bacteria employ a type III secretion system (T3SS) to deliver effector proteins into eukaryotic cells to form mutualistic or pathogenic interactions with their host. A T3SS is comprised of about 25 different proteins many of whose functions still remain obscure. In *Yersinia* Ysc-Yop T3SS, YscX and YscY are two proteins which have not been well characterised. However, a Ysc-Yop T3SS is non-functional in the absence of either *yscX* or *yscY*, suggesting that both YscX and YscY are crucial Ysc-Yop T3SS constituents.

**Objectives**

An attempt to understand the role of YscX and YscY was initially investigated by studying the functional interchangeability between genetically conserved members of the YscX-YscY protein families. It suggested that YscX might be functionally unique to *Yersinia* despite reciprocal binding with YscY family members. Intrigued by the specificity of YscX function, we scrutinized the role of YscX N-terminus in secretion of itself and other T3S proteins.

**Methods**

Site directed mutagenesis and defined domain swapping revealed YscX N-terminus to be a critical aspect of *Yersinia* T3S as it prevented the polymerisation of surface localized YscF needle. This was neither due to a defect in YscX secretion potential nor any observable defect in forming a bipartite interaction with YscY or a tripartite interaction with YscV as measured by standard protein interaction assays.

**Conclusions**

Therefore, the YscX N-terminus must perform dual functions; on the one hand it is a secretion recognition motif and on the other, a non-redundant recognition signal important for the correct assembly of Ysc-Yop T3SS.



**FEMS-2667**

**Bacterial nanomachines**

## **NEW INSIGHTS ON THE ROLE OF SPAS AUTOCLEAVAGE IN SALMONELLA TYPE III SECRETION SYSTEM**

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### **Background**

The type III secretion system (T3SS also known as 'injectisome') is organized in three main structures: a multi-ring basal body that houses the export apparatus, a needle, and a translocon in the host cell membrane. To assemble a functional T3SS, specific substrates must be targeted to the apparatus in the correct order. The substrate specificity switch from early to intermediate substrates occurs once the needle has reached its full length.

The Salmonella T3SS export apparatus protein SpaS, is a multifunctional component involved in the switch. It consists of a transmembrane region and a C-terminal cytoplasmic domain that undergoes autocleavage at a conserved NPTH motif.

### **Objectives**

Elucidating the timing of autocleavage and why it is critical for SpaS function.

### **Methods**

In this study we test expression and secretion levels of T3 proteins by immunoblotting and blue-native gel electrophoresis to study the composition of the assembled T3SS. Our data are complemented by injectisome purification and its visualization by electron microscopy.

### **Conclusions**

Studying autocleavage in its physiological context we could demonstrate that the kinetics of SpaS autocleavage are not regulated by injectisome state of assembly or secretion and that the autocleavage mechanism is independent from needle length control. Rather, autocleavage is a fast process occurring before SpaS incorporation into the injectisome base, which is demonstrated by the fact that a functional injectisomes can assemble on pre-expressed fully cleaved SpaS. We also found that autocleavage at the NPTH motif is not a prerequisite for switching since also SpaS cleavage by an endogenous site-specific protease leads to functional switching.

**FEMS-0962**

**Bacterial nanomachines**

**ISOLATION AND CHARACTERIZATION OF MAGNETOSOMES FROM  
MAGNETOSPIRILLUM GRYPHISWALDENSE AND EVALUATION OF THE  
CYTOTOXIC EFFECT ON EUKARYOTIC CELLS.**

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**Background**

Magnetotactic bacteria are motile prokaryotes with the ability to swim along the Earth's geomagnetic field lines due to the presence of intracellular structures called magnetosomes. Magnetosomes are composed of a mineral core of magnetite or greigite surrounded by a 2-4 nm thick lipid bilayer membrane (1). In recent years, the magnetosomes have attracted great attention because of their potential usefulness in biotechnological and biomedical applications (2).

**Objectives**

In this work, we explore different methods to isolate magnetosomes from *Magnetospirillum gryphiswaldense* and check their biocompatibility on eukaryotic cells.

**Methods**

Two mechanic cell lysis procedures are compared based on the amount and quality of the magnetosomes obtained. The isolated magnetosomes were structurally characterized by TEM as cuboctahedral magnetite particles with an average diameter of 45(±3) nm (3). The aggregation behaviour of magnetosomes in solution is followed by Dynamic Light Scattering technique. The Zeta potential was always lower than -30mV indicating moderately stability. Infrared spectroscopy (FT-IR) revealed the presence of poly-β-hydroxybutyrate under some extraction conditions.

The biocompatibility is evaluated *in vitro* in cytotoxicity assays on murine macrophage ANA-1 cell line. Cells are incubated with 30 pg of magnetite per cell and viability is measured using flow cytometer by annexin-propidium staining. Magnetosomes labeled with fluorescein isothiocyanate are used to track the location of the internalized particles in the cells.

**Conclusions**

No evident signs of cytotoxicity were found after 48 hour of incubation.

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**FEMS-1737**

**Bacterial pathogenicity**

**DETERMINATION OF VIRULENCE OF FOOD-BORNE SALMONELLA  
SEROTYPES BY USING CAENORHABDITIS ELEGANS AS A MODEL SYSTEM**

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**Background**

Salmonellosis caused by *S. enterica* is an important food-borne disease worldwide and pathogenicity and antibiotic resistance of these bacteria is an important health concern. *C. elegans* is a current nematode model to test pathogenicity of *Salmonella*.

**Objectives**

We determined pathogenicity, antibiotic resistance, serotypes and plasmid profiles of 32 non-typhoidal food-borne *Salmonella* isolates and searched for a correlation between these characteristics among the isolates.

**Methods**

Pathogenicity of isolates was tested in *C. elegans* model. Nematodes (20/group) were fed with *Salmonella* isolates grown on NGM agar (0.6 at OD600). The time required for 50% of nematodes in each group to die (TD50) was calculated. *S. Typhimurium* ATCC14028 and *E. coli* OP50 were used as positive and negative controls, respectively. Plasmids were isolated by Kado-Liu method and examined by agarose gel electrophoresis (0.7%). Serotyping of isolates was done by slide agglutination test and their antibiotic resistance was investigated by disc diffusion method.

**Conclusions**

26 of *Salmonella* isolates belonged to Infantis, 4 to Enteritidis, 1 to Kentucky and 1 to Telaviv serotypes. 6 isolates harboured 1-3 plasmids ranging from 1.2 to 35.8 kb. TD50 values were 4.2±0.5 days for *S. Typhimurium* ATCC 14028 fed, 8.0±0.02 days for *E. coli* OP50 fed, and between 3.5-7.2 days for food-borne *Salmonella* fed nematodes. The significance of differences in TD50 values were tested by t test (p<0.05). 22 of isolates were pathogenic in *C. elegans*; 20 of these isolates were multi-drug resistant, 2 were ciprofloxacin resistant and 21 were nalidixic acid resistant. Multi-drug resistant isolates identified as pathogens in nematodes may pose serious health risk for consumers.

**FEMS-2670**

**Bacterial pathogenicity**

## **LECTIN PRODUCTION BY ACINETOBACTER BAUMANNII CLINICAL AND ENVIRONMENTAL ISOLATES**

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### **Background**

*Acinetobacter baumannii* is an emerging opportunistic pathogen which cause severe infections in immunocompromised patients and exhibit resistance to many conventional antibiotics. Factors responsible for *A. baumannii* pathogenesis are numerous; some of them are biofilm formation and hemagglutination, which are dependent on lectin production.

### **Objectives**

The aims were to determine the lectin production ability of the *A. baumannii*, to determine the lectin specificity to different blood types, and to semi-quantify lectin produced by *A. baumannii*.

### **Methods**

The study was carried out with 3 reference strains (ATCC BAA-747, ATCC 19606, NCTC 13423), 8 environmental and 20 MDR *A. baumannii* wound isolates. Lectin production screening was performed using the hemagglutination assay. The supernatant of overnight cultures were examined using 3 % erythrocyte suspension of different blood types (O Rh+, A Rh+ i B Rh+) and results were recorded after 30 min. The semiquantitative determination of lectin production was examined by *ex vivo* modified hemagglutination assay with erythrocyte of A blood type in microplates using two-fold culture supernatant dilutions (from 1/2 to 1/2048).

### **Conclusions**

All examined isolates were able to produce lectins *in vitro* and expressed hemagglutination activity to all examined blood types. The semiquantitative method showed that titer is in a range from 1/4 to 1/64. The clinical strains produced lectins in slightly higher amount compared to the environmental isolates, but without significant statistical difference ( $P>0.05$ ). The results indicate that lectin production can be a new target for development of novel anti-*Acinetobacter* agents.

**FEMS-2886**

**Bacterial pathogenicity**

**MOLECULAR CHARACTERIZATION OF KLEBSIELLA OXYTOCA STRAINS  
ISOLATED FROM PATIENTS WITH ANTIBIOTIC-ASSOCIATED DIARRHEA**

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**Background**

The main complication of colitis would be seen after antibiotic treatment by  $\beta$  lactams, quinolone and aminoglycosides antibiotics. Recently, *Klebsiella oxytoca* has been known to cause this type of diarrhea.

**Objectives**

Investigating the prevalence and characterizations of *K. oxytoca* isolated from patients with antibiotic associated diarrhea. *K. oxytoca* isolates were also tested for cytotoxin production.

**Methods**

This study was carried out from May 2011 to Dec 2013. Fecal samples were collected from hospitalized patients who received antibiotics. Initial cultivation was performed on specific media. Clinical isolates were confirmed by PCR using the specific *K. oxytoca* polygalacturonase (*pehX*) gene. The double disk diffusion test was used to detect ESBLs producing strains. Tracking ESBLs encoding genes were performed by PCR. The bacteria's cytotoxin production was conducted using cell culture on Hep2 cell lines.

**Conclusions**

Five (12%) isolates had cytotoxin activity less than 30%, 12 (30%) strains had moderate cytotoxin activity between 30 and 60% and 23 (58%) strain had cytotoxin activity equal to/or greater than 60%. The cytotoxin producing *K. oxytoca* can be described as one of the causes of antibiotic induced colitis. Drug discontinuation and creating opportunities for the establishment of intestinal normal flora or appropriate medication after antibiogram would perform better chance earlier in patients with hemorrhagic colitis caused by antibiotics.

**FEMS-0781**

**Bacterial pathogenicity**

**DESIGN AND PRODUCTION OF RECOMBINANT PROTEINS OMP P2, OMP P5 AND D15 OF HAEMOPHILUS PARASUIS AND THE STUDY OF THEIR IMMUNITY IN RABBITS**

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**Background**

*Haemophilus parasuis* is the etiological agent of porcine Glässer's disease.

**Objectives**

Based on previous studies, three recombinant outer membrane proteins of *H. parasuis* serovar 5 (P2, P5 and D15) were designed and produced for studying their immunogenicity and potential use as vaccine antigens.

**Methods**

Each gene was amplified and cloned into a pBAD/TOPO vector, this plasmid being enable to express each protein with a tag of histidines for facilitating the subsequent purification. *Escherichia coli* was transformed with the vector and then, it was cultured with arabinose to induce protein expression. As the three proteins were expressed at insoluble fraction, it was necessary to develop a denaturation process using urea, in order to solubilize and refold them. Refolding was performed at the same time as purification by means of immobilized nickel-histidine affinity chromatography. Each of these proteins was retained in the stationary phase and washed with buffers containing decreasing urea concentrations until removing totally urea; the proteins were then eluted with imidazole. Rabbits were used to evaluate the capacity of these proteins to induce a humoral response before testing them in Glässer's disease natural host. Groups of animals were immunized thrice with each of these proteins, and blood was collected after each inoculation. Sera were used to perform indirect ELISAs, in which purified proteins were used as antigen for coating plates.

**Conclusions**

An increasing in antibody titres with seroconversion can be found after immunizations for recombinant P2, P5 and D15 proteins.

**FEMS-1818**

**Bacterial pathogenicity**

**SIDEROPHORE RECEPTOR GENES AS PATHOGENIC MECHANISM IN ADHERENT-INVASIVE ESCHERICHIA COLI STRAINS ISOLATED FROM BIOPSIES OF CHILEAN CROHN'S DISEASE PATIENTS.**

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**Background**

Crohn's disease (CD) is a chronic gastrointestinal inflammatory disease. Adherent invasive *Escherichia coli* (AIEC) strains, which have the capacity to attach and invade intestinal epithelial cells, and also survive within macrophages; have been associated with CD.

**Objectives**

To identify immunogenic proteins in the outer membrane of AIEC isolates obtained from Chilean patients with CD, and determine their role in pathogenicity.

**Methods**

AIECs were isolated from biopsies of Chilean patients with CD. AIEC outer membrane proteins (OMPs) were obtained from cultures grown at 20 °C and 37 °C, and separated by SDS-PAGE. Immunogenic proteins were identified by immunoblot using sera from patients with CD and MALDI-TOF/TOF. Genes coding identified proteins were knocked out and mutant strains were characterized according to: 1) Capacity to adhere and invade intestinal cells, and to survive within macrophages. 2) Differential expression of outer membrane proteins by (2D SDS-PAGE and MALDI-TOF/TOF. 3) Capacity to stimulate production of inflammatory cytokines.

**Conclusions**

Siderophore receptors IutA and ChuA were identified as immunogenic OMPs. No effect in adherence and invasion capacity was observed after knocking out their genes, however, mutant strains did not survive within macrophages. Additionally, mutation altered the outer membrane profile by apparently affecting expression of virulence-associated proteins. A decrease in IL-1, IL-2, IL-12, IL-4 and IFN-gamma levels was detected in supernatants of macrophages infected with the mutant, while TNF- alpha level did not change. Therefore, IutA and ChuA would be required for AIEC intracellular survival in the host, in order to capture iron and express virulence factors. Finally mutating these receptors the strain becomes unstable and avirulent bacteria, which can not survive oxidative stress because other virulence genes are repressed by iron deficiency.



**FEMS-1353**

**Bacterial pathogenicity**

## **H-NS NUCLEOID PROTEIN IS A MASTER REGULATOR WHICH DIFFERENTIALLY REGULATES TYPE 3 PILI AND CAPSULE OF KLEBSIELLA PNEUMONIAE**

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### **Background**

*Klebsiella pneumoniae* (*K. pneumoniae*) is one of the main pathogens in nosocomial infections. Type 3 pilus (T3P) is a virulence factor required for biofilm formation and adherence to epithelial cells. Three promoters control the expression of T3P: *mrkA* (codes for the pilin) *mrkHI* (code for two activator proteins) and *mrkJ* (a repressor protein). H-NS nucleoid protein structures the bacterial genome, mainly Gamma-proteobacteria. Hypermucoviscosity that is attributed to the capsule formation, is a hallmark of pathogenic strains. No reports have been described for the role of H-NS protein in regulating T3P and capsule in *K. pneumoniae*.

### **Objectives**

1. Determine the role of H-NS protein in transcription of T3P.
2. Analyze both the biofilm and capsule formation in the absence of H-NS.

### **Methods**

Transcriptional expression of *mrkA*, *mrkH*, *mrkI* and *mrkJ* genes was carried out by qRT-PCR. Using EMSA, promoter regions (PCR) of *mrkA*, *mrkJ* and *mrkHI* were incubated with purified H-NS protein and DNA-proteins complexes were observed. Biofilm formation was quantified by the method of 96-wells polystyrene microplates. The abundance of capsule was determined by measuring spectrophotometrically the supernatants of bacterial cultures at exponential phase.

### **Conclusions**

*K. pneumoniae hns* mutant affected the growth at exponential phase and resulted in a hypermucoviscosity phenotype. The absence of H-NS affected the biofilm formation and differentially regulated the *mrk* promoters: derepressed and repressed to *mrkHI*-*mrkJ* and *mrkA*, respectively. H-NS directly bound to the three promoters of T3P. The H-NS protein is a crucial regulator of the main virulence determinants of *K. pneumoniae*.

**FEMS-3005**

**Bacterial pathogenicity**

**EXOGENOUS SUPPLEMENTATION WITH BRANCHED-CHAIN AMINO ACID PROMOTES AEROBIC GROWTH OF SALMONELLA TYPHIMURIUM UNDER NITROSATIVE STRESS CONDITIONS**

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**Background**

An intracellular pathogen *Salmonella enterica* serovar Typhimurium can detoxify cytotoxic nitric oxide (NO) produced in phagocytes of host animals by their metabolism depending largely on the flavohemoglobin Hmp. In *hmp* mutant *S. Typhimurium*, NO causes deficiency in amino acids including branched-chain amino acids (BCAA). In the BCAA biosynthetic pathway, iron-sulfur cluster-containing enzymes dihydroxyacid dehydratase (IlvD) and isopropylmalate isomerase (LeuCD) have been implicated to be targets of NO, suggesting the importance of BCAA metabolism in the NO-induced amino acid auxotrophy.

**Objectives**

This study aimed to determine the effect of BCAA supplementation on *Salmonella* resistance to NO under different oxygen conditions, and to test physiological roles of IlvD and LeuCD in the intramacrophage survival of *Salmonella* in the presence or absence of BCAA supplementation.

**Methods**

Gene mutations deficient in *ilvD* and *leuCD* were constructed in *S. Typhimurium*. With mutant *S. Typhimurium* having combinations of these gene mutations and *hmp* mutation, we measured the effect of BCAA on the susceptibility to nitrosative stress, the NO consumption rate, and the intramacrophage growth.

**Conclusions**

Under nitrosative stress conditions, BCAA supplementation restored the growth of *hmp* mutant and mutants further lacking IlvD and LeuCD in aerobic and semiaerobic cultures, but not in anaerobic cultures. Intracellular survival of *ilvD* and *leuCD* mutants in macrophages was also promoted by BCAA supplementation. Results suggest that the NO-induced BCAA auxotrophy of pathogenic bacteria due to inactivation of iron-sulfur enzymes in the BCAA biosynthetic pathway could be rescued by bacterial taking up exogenous BCAA available in oxic environments of host animals.

**FEMS-1978**

**Bacterial pathogenicity**

**PASTEURELLA MULTOCIDA - A NOVEL PARADIGM FOR BACTERIAL INVOLVEMENT IN CANCER**

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**Background**

*Pasteurella multocida* toxin (PMT) is mitogenic for many cell lines. It acts via modification of three of the four families of heterotrimeric G-proteins which are- G<sub>q/11</sub>, G<sub>12/13</sub> and G<sub>i</sub>. PMT has been shown to induce anchorage independent cell growth, enhance anti-apoptotic proteins, increase  $\beta$ -catenin signalling in a differentiating adipocyte system, and up-regulate JAK/STAT and MAPK signalling pathway, all of which are implicated in various cancers. These different effects of PMT suggest it may have a carcinogenic potential.

**Objectives**

Here we are studying PMT as a potential carcinogen by chronically treating cells *in vitro* with PMT to analyse its involvement in cell transformation.

**Methods**

The following end points are being monitored to assess the carcinogenic potential of PMT: cell proliferation, stress fibre formation, cellular morphology, levels of normal G-proteins, levels of PMT-modified G-proteins, and anchorage independent growth. Results achieved so far show that PMT-treated cells have an increased cell number, show morphological changes, and differences in protein levels compared to the untreated cells.

**Conclusions**

Together the data suggests that prolonged exposure to G-proteins modified by chronic treatment with PMT can set cells along a transformation pathway.

**FEMS-1695**

**Bacterial pathogenicity**

**SINC, A TYPE III SECRETED EFFECTOR OF CHLAMYDIA PSITTACI, TARGETS EMERIN AND THE INNER NUCLEAR MEMBRANE OF INFECTED CELLS AND UNINFECTED NEIGHBORS**

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**Background**

*Chlamydia* species encode a type III secretion (T3S) system that enables the translocation of effector proteins into the host cell cytosol, where they target host pathways to benefit the pathogen. We have identified SINC, a novel T3S effector of *Chlamydia psittaci*, a highly infectious avian pathogen, and medically significant zoonotic pathogen of humans.

**Objectives**

To identify and characterize the eukaryotic target(s) of SINC.

**Methods**

Immunofluorescence, immunoelectron microscopy, differential digitonin permeabilization, inhibitors of nuclear transport, BioID (in situ biotinylation analysis of effector-BirA-proximal eukaryotic targets, streptavidin affinity purification, mass spectrometry).

**Conclusions**

SINC uniquely targets the nuclear envelope (NE) of *C. psittaci*-infected cells and uninfected neighboring cells. Digitonin-permeabilization studies of infected or SINC-GFP-transfected HeLa cells indicate SINC targets the inner nuclear membrane (INM). SINC localization at the NE was blocked by importazole, confirming SINC import into the nucleus. Candidate partners were identified by proximity to biotin ligase-fused SINC in HeLa cells and mass spectrometry. Several candidates were identified with high confidence including the nucleoporin ELYS, lamin B1 and four INM proteins (emerin, MAN1, LAP1 and LBR), suggesting SINC interacts with host proteins that control nuclear structure, signaling, chromatin organization and gene silencing. GFP-

SINC association with the native LEM-domain protein emerin, a conserved component of nuclear 'lamina' structure, was confirmed by GFP pull-down. We conclude that SINC is a novel bacterial effector with the capability of globally altering nuclear envelope functions in the infected host cell and neighboring uninfected cells. These properties may contribute to the aggressive virulence of *C. psittaci*.

**FEMS-1962**

**Bacterial pathogenicity**

## **THE ROLE OF BACTERIA AND PUTATIVE TUMOURIGENIC GENES IN THE PATHOGENESIS OF COLORECTAL CANCER**

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### **Background**

Colorectal cancer (CRC) remains one of the most prevalent cancers in the Western world. However, the factors leading to the development of colon cancer have not been determined, with genetics, diet and other environmental factors all likely to play a role. Mechanisms by which *Bacteroides fragilis* and *Fusobacterium nucleatum* may drive colonic tumour development have been identified. Some *B. fragilis* isolates contain a metalloprotease toxin known as BFT or fragilysin. BFT has been demonstrated to promote colonic hyperplasia in animal models. *F. nucleatum* is of relevance due to the consistent finding across several studies that it is enriched on CRC tumours. Its FadA adhesin can mediate invasion into the cell with a resultant increase in expression of host oncogenes.

### **Objectives**

Advances in next generation sequencing technologies have led to a high resolution of the CRC microbial community. The aim of this study is to use a hypothesis led approach to focus on potential key isolates and genes in the development of colonic tumours.

### **Methods**

We are compiling a comprehensive collection of human colonic tissue from patient cohorts representing different stages of disease. These samples are being analysed using a PCR-based assay to determine the prevalence of selected bacterial taxa and genes. Results show primer specificity, PCR optimization and sensitivity of detection that have been experimentally determined.

### **Conclusions**

Overall this work describes the design and validation of a panel of primers with the intention of analysing a fresh collection of human tissue. Preliminary findings using a collection of pilot samples will be presented.

## FEMS-3089

### Bacterial pathogenicity

#### • GENOMIC CHARACTERIZATION OF AVIAN PATHOGENIC AND COMMENSAL CHICKEN *E. COLI* ISOLATES

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### Background

Extraintestinal pathogenic *E. coli* (ExPEC) are a major source of urinary tract infection, newborn meningitis and sepsis in humans. Avian pathogenic *E. coli* (APEC) cause colibacillosis, a complex systemic infection in poultry. Certain human ExPEC and APEC cannot be clearly distinguished based on molecular epidemiology and their virulence-associated genome content. APEC are considered as a reservoir of virulence- and resistance genes for human ExPEC and a zoonotic risk cannot be excluded.

### Objectives

To characterize potential differences between APEC and chicken commensal *E. coli*, we compared the virulence- and antibiotic resistance-associated genome content of commensal *E. coli* isolates from healthy chicken with APEC strains.

### Methods

*E. coli* isolates from tracheal and fecal samples of healthy chicken were collected and subjected to virulence gene and antibiotic resistance profiling. The genomes of 127 commensal and APEC isolates were sequenced.

### Conclusions

The virulence gene content of commensal chicken and APEC isolates was similar. Comprehensive genome comparison of APEC and chicken commensals will extend our knowledge of bacterial traits that could contribute to pathogenesis and antibiotic resistance. Interestingly, animal husbandry has a great impact on the spread of antibiotic resistance determinants among chicken *E. coli* isolates. As APEC have a zoonotic potential and serve as potential gene pool for human ExPEC, the increased presence of antibiotic resistance determinants in *E. coli* from conventionally raised



chicken can facilitate the spread of multiresistant human ExPEC.

**FEMS-1666**

**Bacterial pathogenicity**

**PROPERTIES OF THE DSBA PROTEIN (CLA0559) FROM CAMPYLOBACTER LARI RM2100.**

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**Background**

*Campylobacter spp.* are known to be one of a major cause of gastrointestinal illness throughout the world. Most prevalent species responsible for human infections are *C. jejuni* and *C. coli*. Human infections by *Campylobacter lari* are also associated with gastrointestinal diseases but mostly in developing countries. So far, little is known about *C. lari* molecular attributes. CLA0559 protein shows high homology to members of DsbA family. As Dsb enzymes control the formation and rearrangement of disulfide bonds during the folding of extracytoplasmic proteins they play an important role in pathogenesis. *In silico* analysis indicate that *C. lari* Dsb system might be a novel, different from the ones described for those operating in *E. coli* or *C.jejuni* cells.

**Objectives**

*C. lari* CLA0559 mutant remains motile but becomes sensitive to DTT. *E. coli* complementation tests (cadmium resistance, motility test and alkaline phosphatase assay) clearly showed that CLA0559 substitutes for EcDsbA activity and acts in cooperation with EcDsbB. CLA0559 functions *in vitro* as an oxidase. It was proven by *in vitro* RNase activity assay. CLA0559 doesn't cooperate with CIDsb1/Dsb12, homologs of DsbB).

**Methods**

All genetic manipulations were performed using standard molecular biology procedures. For complementation purposes in *E. coli* cells *CldsbA* was expressed from low copy vector. *In vitro* tests were performed with CIDsbA purified from *E. coli* using affinity and size exclusion chromatography

**Conclusions**

Our results showed that *cla0559* gene encodes for CIDsbA protein functioning in an oxidizing pathway in *C. lari* cells. Its redox partner still remains unknown.

**FEMS-2371**

**Bacterial pathogenicity**

**IN VITRO PROPERTIES OF THE TRUNCATED FORM OF HELICOBACTER  
PYLORI HP0231 PROTEIN.**

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**Background**

Dsb proteins control the formation and rearrangement of disulfide bonds during the folding of membrane and exported proteins. The mechanism of disulfide bond formation in microorganisms is extremely diverse. The *H. pylori* Dsb system seems to be novel and different from the Dsb system in *E. coli*. Dsb system plays an important role in *H. pylori* pathogenesis as *H. pylori* mutant impaired in disulfide bond formation revealed greatly reduced ability to colonize mice gastric mucosa.

**Objectives**

Our recent work led to the characterization of the HP0231 functioning mainly in an oxidizing pathway in *H. pylori* cells. It is intriguing that HP0231 acts as periplasmic oxidase, as EcDsbA, despite its structural resemblance to EcDsbG. Additionally HP0231 acts as chaperone and is involved in the cytochrome c biogenesis. To assess relations between HP0231 structure and its oxidizing activity we tested biochemical activities of the truncated form of HP0231 (HP0231m) containing only catalytic domain.

**Methods**

All genetic manipulations was performed using standard molecular biology procedures. Correctness of the obtained constructs were verified by sequencing. HP0231m was overexpressed in *E. coli* Rosetta strain and purified using NGC™ Medium-Pressure Chromatography (Bio-Rad). Purified protein was used to perform insulin reduction assay, chaperone activity assay and izomerization/oxidation RNase activity test.

**Conclusions**

Our results show that HP0231m mutated protein possess chaperone activity slightly higher than native HP0231 form and at the same time it lost ability to reduce insulin. It displays the same level of oxidation activity as HP0231 and similarly to native HP0231 does not have izomerization activity.

**FEMS-1038**

**Bacterial pathogenicity**

**NOD1+32656 T>GG FUNCTIONAL POLYMORPHISM AFFECTS SUSCEPTIBILITY TO CHLAMYDIA TRACHOMATIS (CT) INFECTION AND RISK OF TUBAL FACTOR INFERTILITY IN WOMEN**

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**Background**

Genital *CT* infections cause tubal factor infertility (TFI) in some women. Bacterial motif recognition by intracellular pattern-recognition receptors NOD1 and NOD2 can trigger immune response. Whether their functional polymorphisms impact *CT* infection has not been researched.

**Objectives**

Our objective was to test the effects *NOD1*+32656 T>GG and *NOD2* 1007fs on susceptibility and severity of *CT* infection in Dutch Caucasian women.

**Methods**

*Susceptibility cohort:* We selected 737 women visiting the Amsterdam STD outpatient clinic. Questionnaires were collected in regards to urogenital complaints.

*Severity cohort:* 490 Dutch Caucasian female patients visiting the UMC Groningen Fertility clinic were selected. Laparoscopy was used to grade the tubal pathology status as TFI grade 0-4. Controls were TFI grade 0 as assessed by either laparoscopy or hysterosalpingography (HSG).

**Conclusions**

The *NOD1* +32656 GG insertion appears protective against *CT* infection, but predisposes to TFI and a higher occurrence of symptoms in women with a past *CT* infection. The GG variant might be enhancing successful clearing while acting deleterious in the upper genital tract, causing tubal pathology.

**FEMS-3130**

**Bacterial pathogenicity**

**GENOTYPIC AND PHENOTYPIC CHARACTERIZATION OF ATYPICAL ENTEROPATHOGENIC ESCHERICHIA COLI (AEPEC) AND ENTEROHEMORRHAGIC E. COLI (EHEC) ISOLATED FROM OVINE IN BRAZIL**

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**Background**

Atypical Enteropathogenic Escherichia coli (aEPEC) and Enterohemorrhagic E. coli (EHEC) are human pathogens that causes attaching and effacing lesions (A/E) in the intestinal mucosa. EHEC differs from aEPEC because it carries stx genes.

**Objectives**

Characterizing genotypic and phenotypic samples of aEPEC and EHEC.

**Methods**

E. coli was isolated from 130 healthy sheep and characterized by PCR technique as aEPEC (eae+/bfpA/stx-) and EHEC (eae+/stx+). 25 samples of aEPEC and 14 samples of EHEC were analyzed by PCR for presence of ehx, tirY-P (Tir phosphorylated), tirS (no phosphorylated), tccp e tccp2. PCR-triplex was used for phylogenetic classification with chuA, vjaA genes and fragments of TspE4.C2. Samples were also tested for sorbitol fermentation (SOR), enzymatic activity of  $\beta$ -D-glucuronidase (GUD), hemolysins production and HEp-2 cell adhesion.

**Conclusions**

The stx gene subtypes analysis by PCR and cytotoxic effect in Vero cells were realized only in EHEC. The ehx, tirY-P, tirS e tccp2 genes frequency was 40, 60, 16 and 16% in aEPEC and 78, 64, 7 and 28% in EHEC. The tccp gene was present only in aEPEC and only two aEPEC samples were SOR-/GUD+, being the another samples characterized as SOR+/GUD+. Enterohemolysin expression was analyzed in 32% of aEPEC and 66% of EHEC. A diversity patterns adhesion were observed. For the phylogeny, both patotypes were classified as belonging to B1 group. A 100% of EHEC samples presented cytotoxic effect in Vero cells, eight (57%) presented stx1c and seven (60%) stx2d. We concluded that aEPEC and EHEC, isolated from ovine, are two heterogenic groups of bacteria.

**FEMS-2035**

**Bacterial pathogenicity**

## **DOMINANT GENOTYPE OF STAPHYLOCOCCUS AUREUS IN A WELFARE CENTER**

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### **Background**

*Staphylococcus aureus* is associated with various diseases both in hospitals and in the community. The MRSA strains had manifested primarily in hospitals, but in recent years have appeared in community outbreaks in healthy people, so is called MRSA strains community-acquired (CA-MRSA), and now is important its detection.

### **Objectives**

Identify MRSA strains in vulnerable adults admitted to a Welfare Center

### **Methods**

Pharyngeal, nose and hands swabs were taken, of 100 volunteers in two samples of 50 people each; one in November 2012 and another in May 2014. The presence of *S. aureus* was determined by microbiological methods and biochemical tests. MIC for oxacillin was performed. Detection of genes for resistance to methicillin, *mecA* and Panton-Valentine Leukocidin, *lukS-PV* / *lukF-PV*, was made by PCR. The strains were typed by *spa*-typing and the clonal determination was performed by PFGE.

### **Conclusions**

44 strains of *S. aureus* were isolated: 15 in throat, 13 in nose, and in 16 hands. Only two strains were resistant to methicillin. The dendrogram obtained from PFGE banding pattern shows that almost all strains are identical, which was corroborated by the *spa*-type where 83% of the strains tested showed the same *spa*-type t003.

One dominant genotype of *S. aureus* in the population studied was found: *spa*-type t003. The MRSA clone of this type is dominant in Europe, but was not reported in Mexico in addition the type found here is methicillin-susceptible (MSSA). It could be a health problem in this community center.

**FEMS-2141**

**Bacterial pathogenicity**

**DETECTION OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS SPP IN ICU AND IN AREA HOSPITAL.**

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**Background**

Nosocomial infections are an important cause of morbidity and mortality worldwide. At present, *Staphylococcus aureus* and *Staphylococcus epidermidis* are important etiologic agents of hospital infections. *S. aureus* is common in surgical areas of adult intensive care (ICU), sepsis being the most common process involved. Currently strains of community-acquired MRSA (CA-MRSA) have entered in the hospitals.

**Objectives**

The aim of this study was to detect the strains of community-acquired methicillin-resistant *Staphylococcus* in a General Hospital

**Methods**

Swabs of oral and nasal cavity of 52 patients, both male and female with different pathologies were taken, aged between 28 and 72 years, 14 patients were in the ICU and 38 the hospitalization area. The presence of *S. epidermidis* or *S. aureus* was determined by microbiological methods. MIC for oxacillin was measured. The presence of the *mecA* gene, the Panton-Valentine leukocidin gene, and the arginine catabolic mobile (ACME) gene, were detected by PCR. The strains were typed by *spa*-type.

**Conclusions**

We found that 100% of the samples analyzed in the area of hospitalization were strains of *S. aureus*, of which 37% were resistant to methicillin (MRSA). While 93% of the strains tested in ICU were *S. epidermidis*, and 31% of these were methicillin resistant (MRSE). All methicillin-resistant strains showed the presence of the *mecA* gene. One CA-MRSA strain and two CA-MRSE strains were found.

We detected the presence of community-acquired methicillin-resistant *Staphylococcus* in the hospital analyzed, so it is important to conduct periodic sampling to prevent outbreaks caused by this pathogen.

**FEMS-2256**

**Bacterial pathogenicity**

**DETECTION OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN A NURSERY**

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**Background**

*Staphylococcus aureus* is a microorganism of great medical importance. For many years it has been recognized as one of the major pathogens for humans. Infections with methicillin-resistant *S. aureus* (MRSA), are usually acquired in hospitals (strains HA-MRSA). However, in the late 90's, MRSA strains emerged in healthy adults and children in communities. These strains cause infections in the community. The prevalence of these infections has increased significantly in recent years. Strains of *S. aureus* that cause these infections are called strains MRSA community-acquired (CA-MRSA).

**Objectives**

The aim of this work was to make the identification and molecular characterization of strains of *Staphylococcus aureus* isolated in a daycare of Mexico City.

**Methods**

Throat swabs samples from 87 children, between 2 and 6 years old were taken. *S. aureus* was identified by microbiological methods. The *mecA* and Panton-Valentine leukocidin (PVL) genes were detected by PCR. The *SCCmec* type and *spa*-type were determined.

**Conclusions**

25% of the population had *S. aureus* in the throat. From the strains isolated, we found that 22% (five strains) were MRSA, of which only one present the *mecA* gene, the gene of PVL and *SCCmec* type IV, therefore we found a CA-MRSA strain. The results show there may be strains CA-MRSA in healthy carriers in Mexico City, so strains CA-MRSA are present circulating in the community.



**FEMS-2312**

**Bacterial pathogenicity**

**PRESENCE OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN AN ORPHANAGE**

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**Background**

*Staphylococcus aureus* is a pathogenic bacterium that is associated with various diseases both in hospitals and in the community. Between 20% and 35% of the adult population are carriers of this microorganism in the nasal vestibule, but can also be found in the pharynx of people. The MRSA strains had manifested primarily in hospitals, but in recent years have appeared in community outbreaks in healthy children and adults, with no history of hospitalization. These strains were called community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA).

**Objectives**

The objective of this work was to detect the presence of strains CA-MRSA in an orphanage in Mexico City.

**Methods**

Pharyngeal and nasal swabs of 80 people between 3 and 19 years old of both genders were taken. We identified *S. aureus* by microbiological methods. The *mecA* and Panton-Valentine leukocidin (PVL) genes were detected by PCR. The *SCCmec* type and *spa*-type were determined.

**Conclusions**

56% of the population had *S. aureus*; 12.5% only in the pharynx, 17.5% only in the nose and 26% at both sites. We detected only four MRSA strains (5%), one in the pharynx and three in the nose, which presented the *mecA*, the PVL genes, and have the *SCCmec* type IV, so they are CA-MRSA strains. The results show that there are CA-MRSA strains in healthy children and adolescents in an orphanage in Mexico City.

**FEMS-1390**

**Bacterial pathogenicity**

**IDENTIFICATION AND REGULATION OF A NOVEL CITROBACTER RODENTIIUM GUT COLONIZATION FIMBRIAE (GCF)**

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**Background**

*Citrobacter rodentium* is a natural mouse bacterial pathogen that has been extensively used as a surrogate model for studying the human pathogens enteropathogenic and enterohemorrhagic *Escherichia coli*. During infection these pathogens employ surface structures called fimbria to adhere and colonize the host intestinal epithelium; however, for *C. rodentium* only a small number of its fimbrial operons have been studied.

**Objectives**

To analyze the regulation and role in colonization of the *C. rodentium* fimbrial operon *gcfFGABCDE*.

**Methods**

The role in colonization of the Gcf fimbriae was evaluated in mouse colonization assays, while the regulation of the *gcf* operon was assessed using transcriptional fusions and western blot. The promoter of *gcf* was identified by *in silico* analysis, primer extension assays and site-directed mutagenesis. Mutant strains and electrophoretic mobility shift assays were used to determine the role of H-NS as a repressor of *gcf* expression.

**Conclusions**

Here we report the characterization of Gcf (Gut colonization fimbriae) as an important *C. rodentium* colonization determinant of the mouse gastrointestinal tract. We demonstrated that the promoter of the *gcf* fimbrial operon is highly repressed under several *in vitro* growth conditions by H-NS1, one of the five H-NS paralogs encoded in the *C. rodentium* genome. H-NS binds to the regulatory region of *gcf*, further supporting its direct role as a repressor. The *gcf* operon possesses novel and interesting features that open future opportunities to expand our knowledge of these

essential bacterial structures during infection. Work supported by grants IN209713 (DGAPA) and 154287 (CONACyT).

**FEMS-2245**

**Bacterial pathogenicity**

**SALMONELLA TYPHIMURIUM STRAINS WITH DISTINCT CLINICAL PHENOTYPES CAN BE DIFFERENTIATED AT THE TRANSCRIPTOMIC LEVEL**

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**Background**

*Salmonella* Typhimurium infects a wide range of animal hosts, and generally causes a self-limiting gastroenteritis in humans. However, some variants of this serovar, sequence-type ST313, have been associated with an emergent invasive *Salmonella* disease in sub-Saharan Africa, which usually affects susceptible HIV<sup>+</sup> or malarial individuals. A genomic comparison between an ST313 isolate, D23580, and the well-characterized 4/74 strain (sequence-type ST19) shows that both strains share 96% coding genes. Genetic differences include 1000 SNPs, D23580-specific prophages and the presence of pseudogenes.

**Objectives**

We tested the hypothesis that the two strains had different pathogenic mechanisms that are reflected by altered gene expression patterns and gene fitness in environmental conditions that reflect the infectious process.

**Methods**

RNA-seq-based transcriptomic data were obtained for strains 4/74 [1] and D23580 grown under seventeen infection-relevant *in vitro* conditions. Transcriptomic data were validated with a proteomic approach. In addition, a transposon library was generated in the D23580 strain and sequenced after passaging in different media.

**Conclusions**

Comparative transcriptomics between the two strains revealed that the proportion of differentially-expressed genes varied between 1% to 9% of all genes in various stress conditions. Proteomics for the early stationary phase condition confirmed 60% and 54% of the transcriptomic data in 4/74 and D23580, respectively. We are currently analysing the D23580 transposon library data. The differences observed in the expression and fitness of virulence-associated genes under specific environmental conditions may reflect altered regulatory mechanisms of these *Salmonella* strains.

**References:** <sup>1</sup>Kröger *et al* (2013) *Cell Host Microbe* 14: 683-95.

**FEMS-1961**

**Bacterial pathogenicity**

## **ADHERENCE PROFILE OF ATYPICAL ENTEROPATHOGENIC ESCHERICHIA COLI (AEPEC) STRAINS ISOLATED FROM DOGS IN BRAZIL**

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### **Background**

Atypical Enteropathogenic *E. coli* (aEPEC) is a human pathotype implicated in endemic infantile diarrhea. aEPEC with an identical virulence gene profile has been isolated from several animals including dogs, suggesting a zoonotic behavior. Adherence to host enterocytes is a key step of aEPEC virulence, being mediated through the expression of LEE locus genes, leading to formation of attaching-effacing (A/E) lesions. However, similar to LEE-positive Shiga Toxin-producing *E. coli* (STEC), aEPEC may contain distinct non LEE-associated effectors molecules and adhesins.

### **Objectives**

Investigate the adhesion profile of aEPEC strains through search of non LEE-associated virulence markers and interaction assays with epithelial cells.

### **Methods**

We analyzed by PCR the distribution of several virulence genes, including 16 *nle* genes, among aEPEC strains isolated from children (n=5) and dogs (n=5). Adherence phenotypes were determined through *in vitro* assays with HEp-2, Caco-2 and T84 cells.

### **Conclusions**

The occurrence of virulence genes was: *ecpA* (100%), *fimH* (80%), *lpfA* (60%), *hcpA* (50%), *toxB* (30%), *iha* (10%). Fifteen *nle* genes were detected in all strains with different combinations. aEPEC of O157:H7 and O4:H16 serotypes carried the *nleB* and *nleE* genes, both located in PAI-122, an important pathogenicity island of STEC O157:H7. All strains showed a localized-like adherence phenotype (LLA) in HEp-2, Caco-2 and T84 cells. Although aEPEC strains are a very heterogeneous group, these findings show a similar virulence profile between human and canine isolates belonging to the same serotype, suggesting an important role for dogs in the maintenance of the virulence gene pool of aEPEC.

**FEMS-2230**

**Bacterial pathogenicity**

**PRESENCE OF L-ASPARAGINASE II (ANSB) IN OUTER MEMBRANE PROTEIN EXTRACTS OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) AND ITS ROLE IN PATHOGENICITY.**

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**Background**

L-asparaginase II (AnsB) enzyme of *E. coli*, known for their use in treatment of children with acute lymphoblastic leukemia (ALL), and has been described as a bacterial periplasmic protein. However, we detected its presence in outer membrane proteins (OMP) extracts of different STEC serotypes, by Western blot using sera from STEC infected-patients and diagnosed with HUS. Recent reports using a mouse T cells co-cultured with wild type *S. Typhimurium* strain, showed that AnsB is sufficient to suppress T lymphocyte blastogenesis.

**Objectives**

Determine the AnsB presence in outer membrane protein extract of different STEC serotypes, its secretion and effect on T lymphocyte proliferation

**Methods**

The OMP and soluble proteins fractions were separated by SDS- PAGE 12% and western blot directed to AnsB detection was performed with commercial specific antibody anti-AnsB. A electron microscopy using a secondary antibody labeled with gold particles for visualization by immunogold was done. Peripheral blood mononuclear cells, from healthy individuals were incubated in the absence or presence of STEC O157: H7, O157:H7 $\Delta$ ansB, O157:H7 $\Delta$ ansB/pVB1\_ansB, STEC O113:H21, commensal *E. coli* HS strain and *S. Typhimurium*, as a negative and positive control for AnsB effect, respectively. The suppression of proliferation was measured by flow cytometry. Study protocols for peripheral-blood mononuclear cells were approved by the Institutional Review Board of the Faculty of Medicine, University of Chile.

**Conclusions**

We observed by immunogold that the AnsB enzyme is secreted and some molecules remain associated with the outer membrane in STEC. AnsB expressed in O157: H7 contribute to its pathogenicity by inhibiting T lymphocyte proliferation.

**FEMS-0850**

**Bacterial pathogenicity**

## **INSIGHTS INTO THE AUTOTRANSPORT PROCESS OF TRIMERIC AUTOTRANSPORTER YAD A.**

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### **Background**

YadA is a trimeric autotransporter adhesin present in *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*. It has a transmembrane domain which forms a highly stable  $\beta$ -barrel anchoring it to the outer membrane and an N-terminal passenger domain which translocates through the  $\beta$ -barrel forming a coiled-coil stalk followed by a sticky head which mediates attachment of bacteria to host cell ECM components (1). The mechanism by which the N-terminal domain is translocated is still under debate. A relatively flexible region has been found near the C- terminus of the passenger domain known as the ASSA region which has been proposed to act as a hairpin during translocation (2).

### **Objectives**

The idea is to mutate residues in the 'ASSA' region to obtain stalled translocation intermediates. The intermediates will be analyzed with various methods, followed by structural analysis using solid state NMR experiments.

### **Methods**

**Heat stability assays** of the mutants indicated a different tertiary structure as well as less stability compared to wildtype. **Infrared Spectroscopy** indicated loss of  $\alpha$ -helical content of mutant YadA by 33%. **Electron Microscopy** images showed that mutant YadA does not form fibre-like projections on cell surface and cells do not autoaggregate which was proved in **Autoaggregation assays**.

### **Conclusions**

Preliminary results indicate a translocation intermediate which needs to be further analysed by ssNMR.

### **References**

1. El Tahir Y, Skurnik M. (2001).YadA, the multifaceted Yersinia adhesion. Int J Med Microbiol. 291(3):209-18.
2. Shahid SA et al. (2012) Membrane-protein structure determination by solid-state NMR spectroscopy of microcrystals. Nat Methods 9, 1212-1217.



**FEMS-3041**

**Bacterial pathogenicity**

**FUNCTIONAL GENETIC ANALYSIS OF THE ENVZ-OMPR TWO-COMPONENT SYSTEM IN ENTEROHEMORRHAGIC E. COLI PATHOGENICITY**

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**Background**

Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) causes severe diarrhea, hemorrhagic colitis, and can also lead to life-threatening diseases like hemolytic uremic syndrome (HUS). Several EHEC virulence factors have been reported and encoded in the virulence plasmid pO157 and the O157 pathogenicity islands (O-Islands), including the locus of enterocyte effacement (LEE) island (148 O-Island encodes the type three secretion system and effector proteins) and the 93 and 45 O-Islands (encode the two Shiga-like toxins).

**Objectives**

In our previous studies, we utilized the model animal *C. elegans* for studying EHEC infection *in vivo*. Using this EHEC-*C. elegans* model to perform an EHEC transposon library screen, we found that mutations in the *ompR* gene confer the virulence-attenuated phenotype of EHEC against *C. elegans* animals. Here, we aimed to test whether the EnvZ-OmpR two-component system can regulate these reported EHEC virulence genes to infect *C. elegans*.

**Methods**

We identified several potential OmpR binding sites in the promoter regions of these EHEC virulence genes through *in silico* analysis. Furthermore, the mRNA transcript levels of these EHEC virulence genes were significantly down regulated in the *ompR* deletion mutant.

**Conclusions**

Taken all together, our current data suggested that OmpR is one of the EHEC virulence master regulators to manipulate the expression of virulence factors during infection *in vivo*.

**FEMS-0524**

**Bacterial pathogenicity**

## **FUNCTIONAL ANALYSIS OF BIOCONTROL BACTERIUM PSEUDOMONAS TAIWANENSIS**

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### **Background**

*Pseudomonas taiwanensis* is a broad host range Gram-negative bacterium. Recently, we found that *P. taiwanensis* displayed strong antagonistic activity against rice pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo). Rice bacterial blight caused by Xoo is one of the most destructive diseases of rice worldwide.

### **Objectives**

Understanding which genes are required by *P. taiwanensis* to resist the leaf blight of rice pathogen *Xanthomonas oryzae* pv. *oryzae* (Xoo)

### **Methods**

Here we combined whole genome sequencing and Tn5-transposon mutagenesis to identify anti-Xoo toxin factors and related regulatory pathway. A high quality complete sequencing was accomplished by a combination of Roche 454, Illumina Solexa, Sanger sequencing and Optical mapping.

### **Conclusions**

The complete sequence of the 5.08-Mb genome sequence and 4666 CDS were determined. We used transposon random insertion to identify genes involved in the production and regulation of anti-Xoo activity based on analyses of 6000 individual insertion-strains. Our results show that the siderophore pyoverdine biosynthetic gene (*pvd*), Type VI secretion system (T6SS), and EnvZ/OmpR two-component system have important roles in antagonistic activity against Xoo. On the other hand, the toxicity of *P. taiwanensis* was negatively regulated by the RpoS sigma factor. We further used MALDI-imaging mass spectrometry (MALDI-IMS) to track pyoverdine in *P. taiwanensis* and mutants. The results showed that pyoverdine was positively regulated by EnvZ/OmpR two-component system and secreted by T6SS. In contrast, pyoverdine was negatively regulated by RpoS. To the best of our knowledge, this is the first report that Pyoverdine has toxicity toward Xoo and T6SS can secrete small compounds.

**FEMS-1199**

**Bacterial pathogenicity**

**ISORHAMNETIN INHIBITS PREVOTELLA INTERMEDIA  
LIPOPOLYSACCHARIDE-INDUCED PRODUCTION OF INTERLEUKIN-6 IN  
MURINE MACROPHAGES VIA ANTI-INFLAMMATORY HEME OXYGENASE-1  
INDUCTION**

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**Background**

IL-6 is a key proinflammatory cytokine which has been considered to be important in the pathogenesis of periodontal disease. Host modulatory agents directed at inhibiting IL-6, therefore, appear to be beneficial in terms of attenuating periodontal disease progression and potentially improving disease susceptibility.

**Objectives**

In the current study, we investigated the effect of flavonoid isorhamnetin on the production of IL-6 in murine macrophages stimulated with LPS from *P. intermedia*, a pathogen implicated in inflammatory periodontal disease, and its mechanisms of action.

**Methods**

LPS from *P. intermedia* ATCC 25611 was isolated by using the standard hot phenol-water method. Culture supernatants were collected and assayed for IL-6. We used real-time PCR to quantify IL-6 and HO-1 mRNA expression. HO-1 protein expression and levels of signaling proteins were monitored by immunoblot analysis. DNA-binding activity of NF- $\kappa$ B was analysed by using the ELISA-based assay kits.

**Conclusions**

Isorhamnetin significantly down-regulated *P. intermedia* LPS-induced production of IL-6 as well as its mRNA expression in RAW264.7 cells. Isorhamnetin upregulated HO-1 expression at both gene transcription and translation levels in *P. intermedia* LPS-activated cells. In addition, inhibition of HO-1 activity by SnPP blocked the inhibitory effect of isorhamnetin on IL-6 production. Isorhamnetin failed to prevent LPS from activating either JNK or p38 pathways.

Although further research is required to clarify the detailed mechanism of action, we propose that isorhamnetin may contribute to blockade of the host-destructive

processes mediated by IL-6, and could be a highly efficient modulator of host response in the treatment of inflammatory periodontal disease.

**FEMS-2947**

**Bacterial pathogenicity**

**NON-K1 SEROTYPES OF KLEBSIELLA PNEUMONIAE CAUSING LIVER ABSCESS AND ORGAN COMPLICATIONS IN HEALTHY MICE MODEL**

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**Background**

*Klebsiella pneumoniae* is an opportunistic pathogen that affects immunocompromised patients and commonly associated with nosocomial infections. In the past two decades, the capsular serotype K1 has emerged as the predominant pathogen in causing liver abscess, affecting primarily Asians. While many studies have been reported for K1 and K2 which has comparable virulence to K1, very few of the 82 capsular serotypes have been studied.

**Objectives**

To determine the association between *K. pneumoniae* serotypes K1, K11, K19 and K31, and liver abscess and complications in kidney, spleen and lungs

**Methods**

Bacterial suspensions of the four serotypes of *K. pneumoniae* were inoculated intraperitoneally into healthy mice models. After 72 hours, the organs were aseptically removed, and histopathological examination and bacterial enumeration were carried out. The presence of liver abscess and complications in the other organs were characterized by the observation of necrosis and severity of inflammation, respectively.

**Conclusions**

From the histopathology examination, serotypes K1, K19 and K31 resulted in liver abscess, unlike K11. The spleen was severely inflamed with K1 and mildly inflamed with the other three serotypes. Moderate inflammation was observed in the lungs infected with K1 while the other serotypes caused mild inflammation. The immune system of the mice was able to clear the bacteria in all the organs within 72 hours when infected with all four serotypes. Our preliminary data supports a statistical association between non-K1 *K. pneumoniae* serotypes and liver abscess and organ complications in healthy mice models.



**FEMS-2223**

**Bacterial pathogenicity**

**THE EFFECTOR PROTEIN SDHA FROM PISCIRICKETTSIA SALMONIS IS OVEREXPRESSED DURING AN INFECTIOUS PROCESS IN CELL LINE SHK-1.**

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**Background**

*Piscirickettsia salmonis* is the etiological agent of Piscirickettsiosis disease in Chilean Salmon farming and cause significant economic losses in this industry. For similar pathogens like *Legionella pneumophila* and *Coxiella burnetii* some effector proteins were described that modify cellular processes of the host to establish intracellular infection. Nowadays there is no evidence for similar effector proteins of *P. salmonis*. Only one report described a type 4b secretion system (SST4b) in this pathogen.

**Objectives**

Evaluate gene expression levels of *dotH*, *dotG* and *sdhA* genes during an infectious process induced by *Piscirickettsia salmonis* on SHK-1 cell line.

**Methods**

RNA extraction was performed combining Trizol with commercial kit for total RNA. RT reaction was performed with M-MLV and Real Time PCR was performed by commercial SYBR Green master mix kit.

**Conclusions**

The expression levels of *dotG*, *dotH* and *sdhA* genes are increased in a correlated manner between them during an infectious process induced by *Piscirickettsia salmonis*. At early times such 2 hours after infection the expression levels are increased and then decrease a few fold-change to increase again at the final days of infection but not as much as in the beginning of the experiment. From this pattern of gene expression we propose that the SST4b is involved in traslocating the effector protein SdhA to the cytoplasm of the infected cell.

**Funds**

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**FEMS-1983**

**Bacterial pathogenicity**

**LIPL21 LEPTOSPIRAL PROTEIN BINDS EXTRACELLULAR MATRIX(ECM) COMPONENTS B2 INTEGRINS AND C3/C4 COMPLEMENT FACTORS**

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**Background**

Leptospirosis is a zoonosis with global distribution recognized as a re-emergent disease caused by pathogenic bacteria of the genus *Leptospira*. LipL21 is major leptospiral surface protein identified by proteomics.

**Objectives**

The goal of this work is to obtain the recombinant protein LipL21 expressed in *E. coli* and to evaluate its binding activity with extracellular matrix components (ECM), integrins cell receptors ( $\alpha$ L $\beta$ 2,  $\alpha$ M $\beta$ 2) and C3b and C4b complement factors.

**Methods**

The gene LIC10011 encoding for LipL21 was cloned and expressed in *Escherichia coli*. Protein attachment to individual ECM components,  $\alpha$ L $\beta$ 2,  $\alpha$ M $\beta$ 2 integrins, and C3b and C4b was screened by ELISA, and the binding was evaluated by probing the reaction with anti-LipL21 serum. Binding of LipL21 recombinant protein to ECM and integrins was compared with their binding to gelatin, BSA and fetuin negative controls, by using Student's two-tailed t-test.

**Conclusions**

LipL21 was expressed in *E. coli* with a 6X HIS sequence tag at N-terminal. The protein was present in its soluble form, and was successfully purified in Ni<sup>2+</sup>-charged resin, as assessed by SDS-PAGE. Binding of LipL21 was statistically significant with cellular fibronectin, collagen IV, laminin and  $\alpha$ L $\beta$ 2,  $\alpha$ M $\beta$ 2 integrins, which may be justified by the occurrence of *DGEA* integrin binding domain in LipL21 protein. The binding of LipL21 with C3b and C4b was significantly enhanced when the protein was associated with  $\alpha$ M $\beta$ 2 integrin, probably due the presence of binding sites for these components in the ligand. These results suggest that this protein has the potential to cooperate in the bacterial immune evasion and dissemination in the hosts.



**FEMS-1902**

**Bacterial pathogenicity**

## **CHARACTERIZATION OF VIRULENCE FACTORS IN ESCHERICHIA COLI ISOLATES FROM HAEMOCULTURES**

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### **Background**

Extraintestinal pathogenic *Escherichia coli* (ExPEC) is one of the main etiological agents of Gram-negative bloodstream infections. Capacity of ExPEC to cause systemic infections is due to genes encoding virulence factors, which include adhesins, toxins, polysaccharide coatings, siderophores or iron acquisition systems.

### **Objectives**

The aim of our study was to analyse the presence of virulence genes *fimA*, *bfpA*, *pap*, *sfa*, *eae*, *afa*, *cnf1*, *α-hly*, *aer*, *ial*, *lt*, *st*, *ipaH*, *iucC*, *cdtI-V* and to determine the phylogenetic origin of *E. coli* strains isolated from septicemic patients.

### **Methods**

This study included 133 *E. coli* strains isolated by haemoculture from adult patients with bacteriemia. Automated blood culture system (Bactec 9050) was used in haemocultivation. *E. coli* strains of positive haemoculture were identified on a Microflex MALDI Biotyper (Bruker Daltonik) and confirmed by standard biochemical methods ENTEROtest 16, ENTERO-Rapid 24 (Lachema, Czech Republic). Polymerase chain reaction was used to detect virulence genes and to determine ECOR phylogenetic classification of *E. coli* strains.

### **Conclusions**

We found that *fim A* (96%), *aer* (75%) and *iucC* (68%) represent most common genes encoding virulence properties. Toxin studies revealed a relatively high incidence of *cnf1* (20%) and *α-hly* (19%). Phylogenetic classification showed that 80% of *E. coli* strains tested fall into phylogenetic groups B2 and D, representing main groups of virulent *E. coli*.

## **FEMS-2019**

### **Bacterial pathogenicity**

#### **GENOMIC ANALYSIS TO FIND NOVEL USHER-CHAPERONE ASSEMBLED FIMBRIAE IN ENTEROTOXIGENIC ESCHERICHIA COLI STRAINS**

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### **Background**

Enterotoxigenic *Escherichia coli* is leading cause of diarrhea, mainly in children and travelers to endemic regions. Currently, there is no effective vaccine to prevent ETEC-caused diarrhea. ETEC colonizes the small intestine by using a diverse set of adhesins including about twenty different pili. However, between 15-50% of the isolates obtained from diarrhea cases worldwide are negative in detection of the known adhesins, suggesting presence of additional unidentified adherence determinants and, at the same time, presenting an obstacle in the development of adhesin-based vaccine candidates.

### **Objectives**

To identify novel fimbrial loci in ETEC strains negative for detection of the known adhesins

### **Methods**

Genomes of 35 ETEC strains isolated from diarrhea cases in Chile (14 isolates), Kenya (5), Mozambique (2), The Gambia (1), Mali (3), Bangladesh (3), India (2) and Pakistan (5), which were negative in detection of 23 adhesin genes, were partially sequenced by a paired-end protocol (Illumina, HiSeq 2000). Genomic sequences were screened for 161 fimbrial usher genes belonging to nine families of fimbriae (alpha, beta, gamma-1, gamma-2, gamma-3, gamma-4, kappa, sigma and pi), in order to locate homologous genes and find putative novel loci encoding usher/chaperone assembled adhesins. Genomic sequences of non- pathogenic *E.*

*coli* strains (13) and other ETEC strains (H10407, E24377A and B7A) were included as controls.

### **Conclusions**

Loci encoding putative novel gamma-2 fimbriae were found in 23 strains, being frequent among ETEC strains negative for detection of known adhesins.

Acknowledgements: Fondecyt Grants 3130555 and 1110260. Bill and Melinda Gates Foundation Grants ID 38874 and 1016839.

FEMS-1018

## Bacterial pathogenicity

### COORDINATED REGULATION OF THE MAJOR VIRULENCE GENE *pelD* OF THE PHYTOPATHOGEN BACTERIUM *DICKEYA DADANTII* BY FIS, CRP AND H-NS

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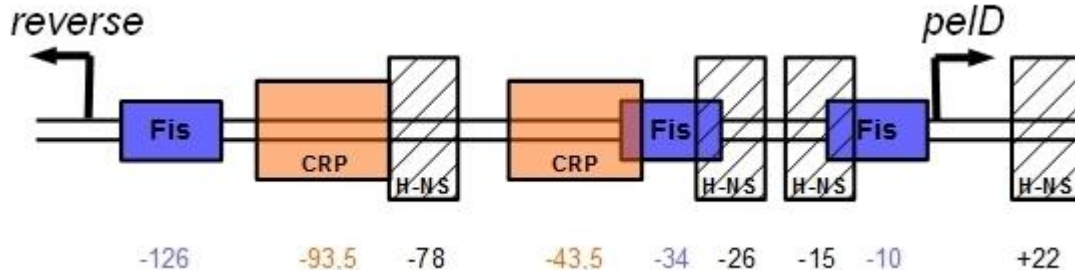
<sup>1</sup>UMR5240 CNRS/INSA/UCB, Université de Lyon, Villeurbanne, France

#### Background

Pathogenic bacteria tightly regulate their virulence genes in response to the variations of their environment. Many transcription factors have been identified for such genes, but their interactions remain poorly understood. The *pel* genes of the phytopathogen bacterium *D. dadantii* are a good example of this situation, with a dozen identified regulators, yet an integrative view of their action was lacking.

#### Objectives

As a first step towards an integrative model, this work aims at taking into account the interactions between the major global regulators of virulence (Fis, CRP and H-NS) in the regulation of the *pelD* virulence gene. In particular, Fis has 2 sites overlapping the promoter (-34 and -10 from the transcription start site) and an upstream site (-126). The relationships between those sites are investigated here as well.



#### Methods

Binding sites were inactivated individually by site-directed mutagenesis and the impact of the modifications was evaluated both *in vivo* by *gfp* gene fusion measurements, and *in vitro* by quantitative DNase I, potassium permanganate footprinting and *in vitro* transcription.

#### Conclusions

*D. dadantii* has established 3 redundant mechanisms to ensure a full repression of *pelD* in exponential phase : 2 of the Fis binding sites (-34 and -10) directly compete with RNA polymerase, 1 Fis site (-34) prevents the activator CRP from binding and a Fis-regulated (involving the -126 Fis site) reverse promoter represses the *pelD* promoter. On top of this, H-NS modulates the action of Fis. These results show that the importance of interactions in transcriptional regulation should not be underestimated.



**FEMS-1316**

**Bacterial pathogenicity**

**YQIC IS REQUIRED FOR COLONIZATION, MOTILITY, AND FLAGELLATION OF  
SALMONELLA TYPHIMURIUM, AND INTERLEUKIN-8 AND HUMAN BETA-  
DEFENSIN-3 PRODUCTION IN HUMAN INTESTINAL EPITHELIAL CELLS**

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**Background**

The phenotype of *Salmonella yqiC* gene and its impacts on host responses are little known.

**Objectives**

To investigate whether *yqiC* is responsible for *Salmonella* colonization/invasion, bacterial motility/adherence, and interleukin (IL)-8/human  $\beta$ -defensin-3 (hBD3) production in human intestinal epithelium.

Fig. 1

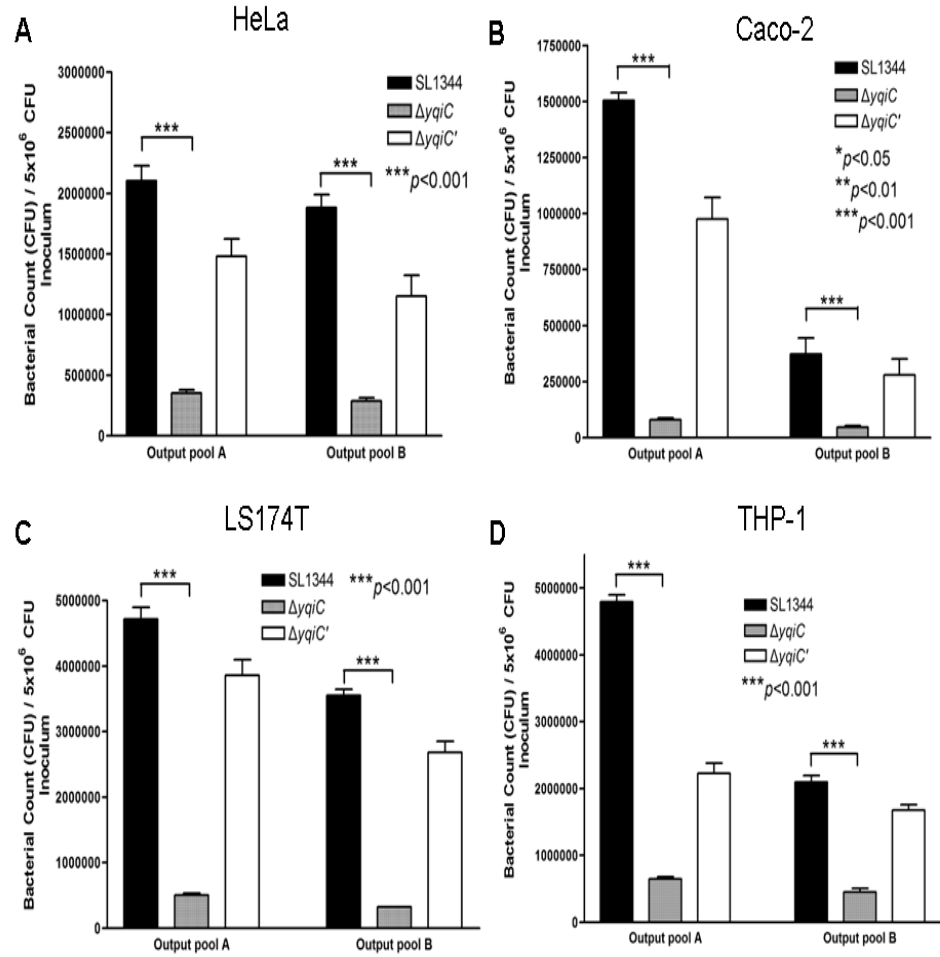


Fig. 2

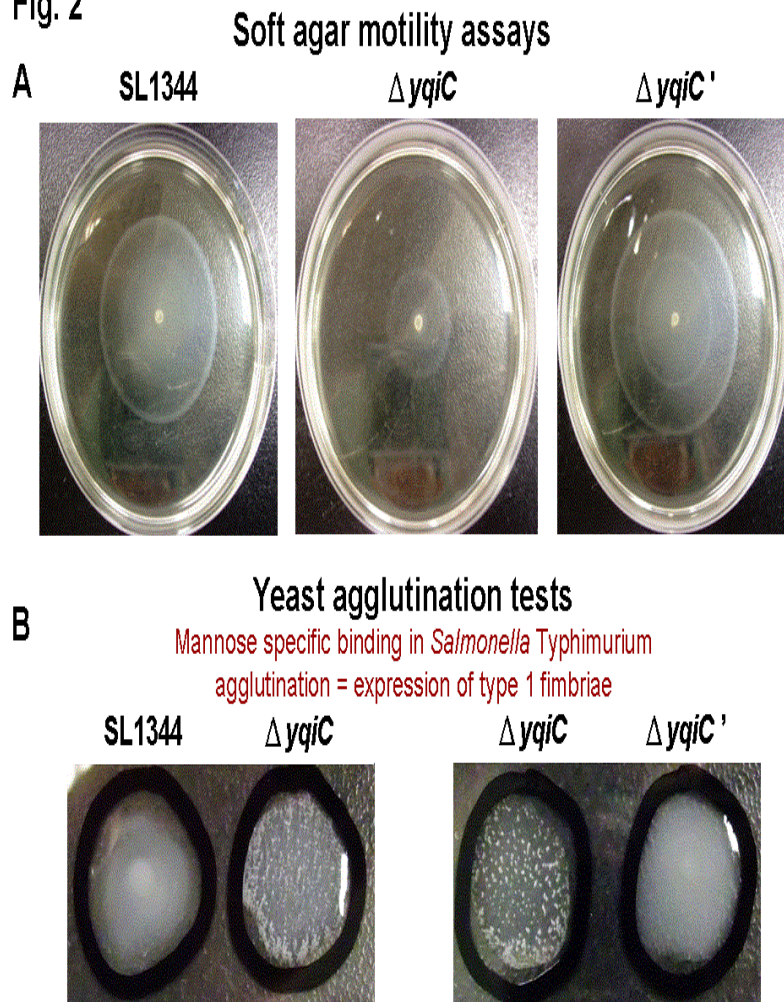
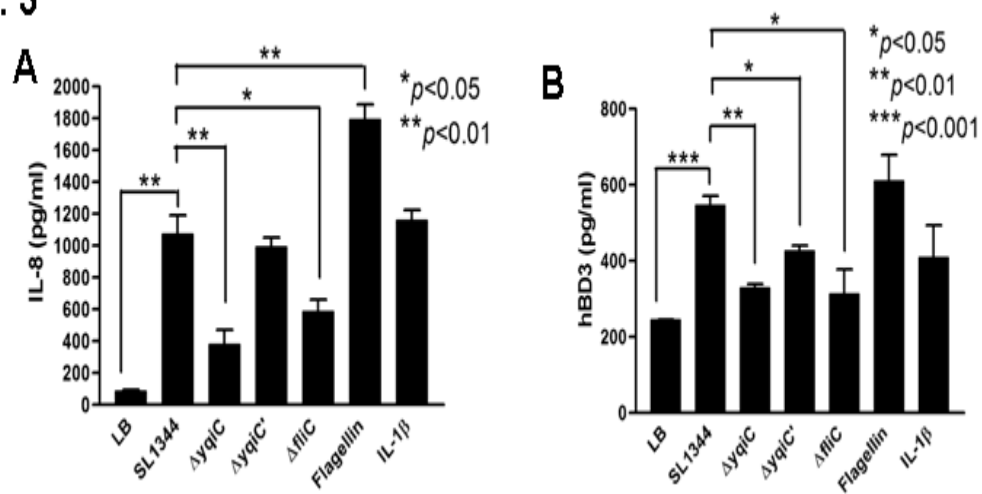


Fig. 3



## Methods

HeLa, Caco-2, LS174T, and THP-1 cells were infected with *Salmonella* Typhimurium



wild-type SL1344, *yqiC*-depleted mutant  $\Delta yqiC$ , its complemented strain  $\Delta yqiC'$ , and *fliC*-deleted mutant  $\Delta fliC$  (MOI=5) for 2 hours, and treated with plain medium (output pool A: colonizing bacteria) or gentamicin (output pool B: invading bacteria) for 1 hour. At these times cell-associated bacterial numbers were calculated. Next, motility and adherence of these strains were examined by soft agar motility assays and yeast agglutination tests. Last, LS174T cells were treated with these strains, flagellin, and IL-1 $\beta$  for 2 hours, incubated in gentamicin for 1 hour, and the infections were continued for 15 hours. At the end-point, the supernatants were collected to measure IL-8 and hBD3 using ELISA.

## **Conclusions**

Compared with SL1344,  $\Delta yqiC$  was attenuated in bacterial colonization and invasion in 4 cells (Fig.1), lost swimming ability (Fig.2A), and constitutively expressed type 1 fimbriae (Fig.2B). IL-8 production in  $\Delta yqiC$ -infected cells was lower than that in SL1344-infected cells, with lower levels than those in  $\Delta fliC$ -infected cells (Fig.3A). Furthermore,  $\Delta yqiC$  and  $\Delta fliC$  induced lower hBD3 secretion than SL1344 did in LS174T cells (Fig.3B).

In conclusion, *yqiC* is required for *Salmonella* Typhimurium colonization, and host IL-8 and hBD3 production. This is mediated by its modulation in *Salmonella* motility and flagellation via downregulation of type 1 fimbriae.

**FEMS-1024**

**Bacterial pathogenicity**

## **ROLE OF FIBRONECTIN IN THE ADHERENCE OF DIARRHEAGENIC *E. COLI* TO INTESTINAL CELLS AND THE INDUCTION OF SECRETION OF PRO-INFLAMMATORY CYTOKINES**

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### **Background**

Fibronectin has been shown to be important for the adherence of diarrheagenic *E. coli* (DEC), but the biological relevance of the DEC-fibronectin-mediated interaction to intestinal cells remains unclear

### **Objectives**

We evaluated the impact of the DEC-fibronectin-mediated interaction on the induction of secretion of pro-inflammatory cytokines

### **Methods**

We assessed the ability of EAEC, ETEC and EHEC to bind to HEp-2 cells in the presence or absence of purified fibronectin protein and we quantified the amount of IL-8 secreted by infected cells. Additionally, we quantified the expression of *FOS*, *NFkB*, *IL8*, *CCL20*, *IL1A* and *TNFalpha* genes. Alternatively, we quantified the IL-8 secretion of HEp-2 cells transfected with small hairpin RNA (shRNA) for fibronectin or scrambled shRNA infected with EAEC strain.

### **Conclusions**

We found a significant increase in the adherence to HEp-2 cells pre-incubated with fibronectin compared with cells not incubated with this protein for all DEC assayed. However, the IL-8 secretion was significantly reduced in the presence of fibronectin. Real-time PCR assay indicated that of all genes assayed only *IL8* gene expression was reduced in HEp-2 cells pre-incubated with fibronectin. Experiments using fibronectin shRNA revealed a decrease in the EAEC adherence to cells, but no changes in the IL-8 secretion.

Overall, all data suggested that fibronectin participates exclusively in the adherence of DEC to epithelial cells, but not in the inflammation originated as a consequence of the infection.



**FEMS-1177**

## **Bacterial pathogenicity**

### **ARO<sup>A</sup> DEFICIENT SALMONELLA – MORE THAN AN AUXOTROPHIC MUTANT?**

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#### **Background**

Attenuated *Salmonella* employed as live vaccine carrier could provide protection against many infectious diseases. To guarantee successful vaccination, balance between attenuation and immunestimulation is required. Since aromatic amino acids are not freely available in the host,  $\Delta$ aroA is commonly used as an attenuating factor. However, we observed that an aroA deficiency also dramatically modified the phenotype of *Salmonella* by increasing bacterial virulence.

#### **Objectives**

In the present work the molecular basis for the phenotypic changes in the strains should be determined by transcriptional profiling and gene deletion of apparently relevant genes.

#### **Methods**

We were able to demonstrate via transcriptome profiling that *Salmonella* is consuming excessive pyruvate by up-regulating sugar pathways like *manXYZ* or *glpABCQT* resulting most likely in an osmotic imbalance. Furthermore, aroA deficient mutants are more prone to express FljB flagella. These metabolic dysregulations could be a possible explanation for the observed phenotype of increased virulence. In accordance after intravenous application of  $\Delta$ aroA *Salmonella* to mice (86/609/EEC), significantly increased induction of pro-inflammatory cytokines like TNF- $\alpha$  or IFN- $\beta$  was observed emphasizing the importance of this deletion for *Salmonella*'s phenotype. We also employed such bacteria in bacteria-mediated tumor therapy. We could show that the  $\Delta$ aroA strains display an increased tumor colonization and an improved anti-tumor response.

#### **Conclusions**

Disturbing the bacterial metabolism by mutating aroA in *Salmonella* leads to a modified phenotype that results on one site in attenuation but also improves *Salmonella*'s ability to induce an immune response. Therefore this mutation might be a preferable modification for vaccine and cancer research.



**FEMS-2102**

**Bacterial pathogenicity**

**THE CAPSULE OF CAMPYLOBACTER JEJUNI PREVENTS BINDING TO SIGLEC-1 AND SIGLEC-7**

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**Background**

*Campylobacter jejuni* (*C. jejuni*) is the leading cause of bacterial gastroenteritis worldwide. Besides diarrhoea, an infection with *C. jejuni* can precede the acute and severe polyneuropathy Guillain-Barré syndrome (GBS). Lipooligosaccharides (LOS), present in the outer membrane of *C. jejuni*, cause activation of the innate immune system and sialylation of LOS is critical for the induction of GBS. Sialylated LOS can be recognised by two host immune-receptors, Siglec-1 and Siglec-7. Recent research indicates that *C. jejuni* LOS are not always exposed to the external environment. We hypothesize that *C. jejuni* exploits the polysaccharide capsule as an evasion strategy to prevent immune recognition mediated by Siglec-1 and Siglec-7.

**Objectives**

To determine whether the polysaccharide capsule of *C. jejuni* prevents the specific binding of bacterial LOS to Siglec-1 and Siglec-7.

**Methods**

Capsule and sialic acid transferase knock-out mutants were generated. Bacteria were FITC-labelled, incubated with Siglec-1 or Siglec-7 transfected cells and binding of live or heat-inactivated bacteria was measured using flow cytometry.

**Conclusions**

Absence of the capsule of *C. jejuni* enhanced binding of *C. jejuni* sialylated LOS to Siglecs. For Siglec-1, this was observed for both live and heat-inactivated bacteria. In contrast, for Siglec-7 the difference in binding between wild-type and non-capsulated *C. jejuni* was only observed for live bacteria. Sialic acid mutants showed lower binding to Siglecs. Our results give insight in how the immune system recognizes *C. jejuni* and define the capsule as a bacterial factor that may influence the onset of GBS.

**FEMS-1572**

**Bacterial pathogenicity**

## **CTRA-DEPENDENT BRUCELLA ABORTUS CELL CYCLE REGULATION IN CULTURE AND DURING INFECTION**

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### **Background**

*Brucella abortus* is a facultative intracellular pathogen for mammals. Many aspects of its intracellular trafficking have been investigated in HeLa cells and RAW264.7 macrophages. However very little is known about its cell cycle regulation, except that bacteria are blocked in G1 phase during early hours of infection.

### **Objectives**

The main goal of this project is to investigate the regulon of CtrA, a central transcription factor.

We also decided to investigate a two-component system (TCS) located upstream of a signalling pathway regulating CtrA phosphorylation status. This TCS involves a histidine kinase, PdhS, and a single-domain response regulator, DivK (Hallez).

### **Methods**

We used RSA-Tools (Van Helden) to look for the consensus sequence bound by CtrA in *B. abortus* genome. This *in silico* approach allowed the prediction of a long list of putative CtrA targets. A ChIP-seq analysis confirmed part of these targets. The activity of some of these promoters was monitored by fusing them to a gene coding for an unstable GFP.

We performed *in vitro* kinase assays to test PdhS autophosphorylation and its ability to transfer the phosphate group to DivK.

### **Conclusions**

Promoters bound by CtrA are predicted to control genes involved in cell cycle regulation, such as division, chromosome replication and segregation.

The reporter system showed that the activity of some promoters varies in bacteria grown in rich culture medium according to their cell size, as well as during *B. abortus* intracellular trafficking in HeLa cells.

*In vitro* kinase assays show that PdhS can autophosphorylate and quickly transfer its phosphate group to DivK.

**FEMS-1194**

**Bacterial pathogenicity**

## **THE DUAL BEHAVIOUR OF CARBON STARVATION GENES PROVIDES A LINK BETWEEN METABOLISM AND PATHOGENESIS OF SALMONELLA**

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### **Background**

The ability of the enteric pathogen *Salmonella* to utilize a diverse set of nutrients, allows it to survive in various nutrient limiting conditions, rendering it one of the most successful pathogens. *Salmonella* employs sophisticated machinery to either acquire a specific nutrient from the surrounding environment or modify the host's ecosystem for its own metabolic benefit. Carbon starvation (*cst*) genes represent one such class that are proposed to be expressed during carbon starvation, the most common stress encountered by *Salmonella*. Two *cst* genes, *cstA* and *yjiY*, predicted to mediate peptide utilization, were previously reported to be regulated by the global regulator CsrA and YehU/YehT two-component system in *E. coli*.

### **Objectives**

In *Salmonella*, *cstA* was shown to be required for virulence in *C. elegans*, however, the underlying mechanism remains unknown. The objectives of this study are to establish the role of *cst* genes in metabolism and to understand how they affect *Salmonella* pathogenesis.

### **Methods**

We generated knockout for the genes *cstA* and *yjiY* in *Salmonella* Typhimurium. Phenotype microarray on various nutrient sources along with transcriptome analysis was carried out for wild-type and knockout strains. Virulence was assessed using cell culture and animal models.

### **Conclusions**

Phenotype microarray confirmed the importance of *cst* genes in metabolism of *Salmonella*. Despite the high similarity in their sequences, *cstA* and *yjiY* displayed different effects on motility, adhesion, biofilm forming ability and virulence in the animal model of infection. This study brings forward pleiotropic role of *cst* genes and highlights the crucial cross-talk between metabolism and pathogenesis of *Salmonella*.



**FEMS-1397**

**Bacterial pathogenicity**

## **TRANSCRIPTOMIC ANALYSIS IN SALMONELLA ENTERICA REVEALS PRODUCTS OF THE STD FIMBRIAL OPERON AS GLOBAL REGULATORS OF GENE EXPRESSION**

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### **Background**

Fimbriae are virulence factors in *Salmonella enterica*, playing diverse roles in bacterial adhesion (Humphries et al. *FEMS microbiology letters*, 201:121-125, 2001). Synthesis of Std fimbriae, that are not expressed under laboratory conditions, has been shown to occur in the animal intestine, but the molecular mechanisms involved remain unknown (Weening et al. *Infection and immunity*, 73:3358-3366, 2005). It was previously described that derepression of the *std* operon occurs in DNA adenine methylase (*dam*) mutants and that HdfR, a poorly known LysR-like factor, is necessary for *std* transcription. However, HdfR-mediated activation of *std* transcription occurs only in a fraction of the bacterial population, suggesting the occurrence of either bistable expression or phase variation (Jakomin et al. *Journal of bacteriology*, 190:7406-7413, 2008).

### **Objectives**

This work aims to further understand the molecular mechanisms that might allow expression of the *std* operon inside the animal and to analyze the effect of its expression in *S. enterica*.

### **Methods**

A genetic screen for additional *std* regulators revealed that the *std* operon itself is a positive regulator, and the downstream genes of the operon, *stdE* and *stdF*, are crucial for this control. Surprisingly, transcriptomic analysis revealed that StdE and StdF control a plethora of *S. enterica* loci including genes involved in motility, chemotaxis, biofilm formation, conjugation, and virulence.

### **Conclusions**

StdEF-mediated control may play a role in the crosstalk between motility, adhesion and invasion, considered essential for optimal *Salmonella* infection. If StdEF expression is subjected to phase variation during infection, subpopulation formation can be expected to occur in the animal intestine.

**FEMS-1888**

**Bacterial pathogenicity**

**IDENTIFICATION OF GENES MODULATING THE INNATE IMMUNE RESPONSE OF MAMMARY EPITHELIAL CELLS TO ESCHERICHIA COLI P4**

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**Background**

Despite continuous efforts to reduce its incidence, mastitis remains a major disease in dairy cows. Main mastitis pathogens include *Streptococcus uberis*, *Staphylococcus uberis* and *Escherichia coli*.

Induction of the innate immune response is a key mechanism in the initiation of the host response during infections of the mammary gland in cows by *Escherichia coli*.

*E. coli* P4 is a prototypical mastitis strain isolated from a case of clinical mastitis. Previous studies showed that *E. coli* P4 was virulent in a mouse model of mastitis. Yet, we have found that this strain induced a reduced pro-inflammatory response in mammary epithelial cells (MEC) compared to other *E. coli* isolates.

**Objectives**

Our objective was to identify genes responsible for the low pro-inflammatory response induced in MEC by *E. coli* P4 and determine if this reduced pro-inflammatory response contributed to the virulence of *E. coli* P4 in a murine mastitis model.

**Methods**

By screening a library of 2000 mutants for increased pro-inflammatory response on MEC, we identified a series of mutations that increase the response of MEC to strain P4. The impact of these mutations on the phenotype of *E. coli* P4 will be presented in details and discussed.

**Conclusions**

Altogether, results presented will allow a better understanding of the initial steps of the interaction between mammary epithelial cells and mastitis pathogens. This work will help understand if the low pro-inflammatory response triggered by *E. coli* P4 contributes to its virulence.

**FEMS-1948**

**Bacterial pathogenicity**

## **GARDNERELLA VAGINALIS AS A TRIGGER OF RECURRENT E. COLI UTI**

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### **Background**

Recurrent urinary tract infection (**rUTI**), most often caused by uropathogenic *Escherichia coli* (**UPEC**), is a life altering, potentially life threatening problem for many women. Asymptomatic carriage of UPEC in bladder reservoirs has long been believed to be a source for rUTI. However, the natural triggers of UPEC emergence from these reservoirs have remained mysterious. Women with bacterial vaginosis (**BV**), an imbalance of the vaginal microbiota, are at increased risk for UTI.

### **Objectives**

We sought to address the long-standing idea that UPEC bladder reservoirs seed rUTI and to understand why women with BV are more prone to UTI. Translocation of BV bacteria to the urinary tract is likely to occur during sexual activity, which is often a prelude to rUTI. We tested the hypothesis that bladder exposure to BV-associated bacteria may initiate UPEC emergence from intracellular reservoirs.

### **Methods**

Mice containing UPEC bladder reservoirs were challenged by transurethral inoculation with either PBS (control) or the BV-associated bacterium *Gardnerella vaginalis*. We monitored the consequences of bladder exposure to *G. vaginalis* by 1) enumerating urinary tract bacterial titers, 2) assessing epithelial exfoliation by immunofluorescence and scanning-electron microscopy and 3) RNAseq analysis.

### **Conclusions**

Here we show that *G. vaginalis* triggers bladder epithelial exfoliation and emergence of *E. coli* from intracellular reservoirs. We observed phenotypes consistent with rUTI, including high UPEC titers and neutrophils in urine.. These data strongly suggest that *G. vaginalis* presence within the urogenital microbiota may be a trigger for rUTI and likely contributes to the substantially increased risk of UTI in women with BV.

**FEMS-1179**

**Bacterial pathogenicity**

**THE EFFECTS OF INSULIN AND GLUCOSE ON EXPRESSION LEVELS OF USP, SFA/FOC, CNF 1 GENES IN AN UPEC STRAIN.**

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**Background:** The gene expression of microorganisms and environmental factors are closely related.

**Objectives:** To investigate possible changes in the gene expression levels of virulence genes [*sfa/foc* (S and F1C fimbriae), *cnf 1* (cytotoxic necrotizing factor), and *usp* (uropathogenic-specific protein)] in a uropathogenic *E. coli* standard strain (UPEC C7) in the presence of insulin and glucose.

**Methods:** The UPEC strain was cultured in broth media (tryptic soya broth-TSB) under 5 different conditions: 0.1% glucose, 20µU insulin, 200µU insulin, 0.1 % glucose+200µU insulin, and control TSB. After incubation for 24 hours at 37°C, total RNA isolations were prepared from all five bacteria cultures. Gene expression levels were determined by quantitative PCR. Changes in gene expression were evaluated using the expression levels of 16sRNA as a “housekeeping gene,” according to the Pfaffl equation.

**Conclusions:** The medium containing 0.1 % glucose+200µU insulin had increased expression levels of all three genes in the *E. coli* C7 strain. In contrast, the other media (0.1% glucose, 20µU insulin, 200µU insulin) had a decrease in the expression levels of virulence genes, all compared to control medium.

This investigation shows that some environmental factors, such as glucose and insulin, could determine the pathogenicity of *E. coli* strains.

**FEMS-1323**

**Bacterial pathogenicity**

**A THERMO-REGULATED PROLINE-RICH SURFACE PROTEIN OF ENTEROCOCCUS FAECIUM BINDS TO FIBRINOGEN, FIBRONECTIN AND PLATELETS AND CONTRIBUTES TO BIOFILM FORMATION**

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**Background**

*Enterococcus faecium* is a commensal of the mammalian gastrointestinal tract. Recently, it has become an important nosocomial pathogen, causing infections that are difficult to treat. However, little is known about the mechanisms that *E. faecium* employs to colonize and infect mammals.

**Objectives**

We aimed to clarify the interactions of *E. faecium* with the host and we hypothesized that genes important for colonization and infection, could exhibit temperature-regulated expression control.

**Methods**

We performed a transcriptome analysis of *E. faecium* E1162, during mid-exponential growth at 25°C and 37°C. Furthermore, we functionally characterized a surface protein of *E. faecium*, that was found to be produced at higher levels during growth at 37°C than at 25°C.

**Conclusions**

We found thirty-three genes expressed significantly higher at 37°C compared to 25°C. One of the most highly upregulated genes (4.4-fold), is predicted to encode a peptidoglycan-anchored surface protein. The N-terminal domain of this protein is unique to *E. faecium* and closely related enterococci. The C-terminal domain contains

three proline-rich repeats, leading us to name the protein PrpA for proline-rich protein A. PrpA is a surface-exposed protein, that was maximally produced in exponentially growing cells at 37°C. PrpA is immunogenic as specific antibodies were observed in patients after they suffered from an *E. faecium* bacteremia. Heterologously expressed and purified PrpA was able to bind to fibrinogen, fibronectin and to platelets. Furthermore, a *prpA* deletion mutant was defective in the early stages of biofilm formation. Our data indicate that PrpA may contribute to the pathogenesis of *E. faecium* infections in hospitalized patients.

**FEMS-2523**

**Bacterial pathogenicity**

**THE DENUDATION OF LATERAL PART PROMOTE CAMPYLOBACTER JEJUNI INVASION IN CACO-2 CELLS.**

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**Background**

The intestinal epithelium provides a physical barrier to luminal bacteria. This barrier serves as the first line of defense against bacterial adhesion and invasion.

*Campylobacter jejuni* is a Gram-negative commensal bacterium in domestic animal and cause gastrointestinal foodborne disease in human. *C. jejuni* is able to adhere and invade gut epithelium, which lead cause of acute gastroenteritis in human. It has believed that the function of tight junctions (TJs) in human intestinal epithelium is closely related with invasion of *C. jejuni*.

**Objectives**

The aim of this study was to investigate interaction between *C. jejuni* and host intestinal barrier functions. In this study, we used cultured islands (unpolarized) and sheets (polarized) form Caco-2cell.

**Methods**

Invasion of *C. jejuni* was measured by gentamycin protection assay in Caco-2 cells. Confocal microscopy was used to examine the localization of internalized bacteria. And the most of bacteria were observed on cellular lateral part in islands of cell. Interestingly, *C. jejuni* efficiently invaded from basolateral surface compared with apical surface and intracellular *C. jejuni* was significantly increased in disruption of TJs by the treatment with EGTA. Furthermore, *C. jejuni* infection induced the distribution of the TJs component.

**Conclusions**

These date indicated that the TJs formed by each of neighboring cells might act as physical barrier for prevention of *C. jejuni* invasion, and infection of *C. jejuni* might effect on formation of TJs component. These results suggested that the *C. jejuni* infection inducible TJs disruption might enhance *C. jejuni* invasion into host cell.

**FEMS-1673**

**Bacterial pathogenicity**

## **CAPNOCYTOPHAGA CANIMORSUS IS ADHERING TO HOST MEMBRANE GLYCOPROTEINS**

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### **Background**

*Capnocytophaga canimorsus* (Cc), member of the *Bacteroidetes* phylum, is an oral commensal from dogs which causes rare but severe infections in humans. Cc feeds on sugar moieties of glycoproteins expressed on host cell membranes. Removal of N-linked glycans is achieved by the glycoprotein deglycosylation (Gpd) complex encoded by a polysaccharide utilization locus, a hallmark of *Bacteroidetes*.

### **Objectives**

The aim of this study is to determine whether Cc is adhering to the host cells it is feeding on and, if so, which mechanisms are implicated.

### **Methods**

Cell lines were infected with strain Cc5. Mutant bacteria were constructed by site-directed gene replacements. Adhesion was monitored by microscopy and flow cytometry.

### **Conclusions**

Cc5 adheres to epithelial, endothelial and immune cells. In *Flavobacterium johnsoniae*, another member of the *Bacteroidetes* phylum, gliding motility and adhesion are associated. In Cc5 adhesion was not abrogated in a mutant unable to glide. Adhesion of Cc5 was mainly mediated by the Gpd complex. Cc5 adhered to host cells via the membrane glycoproteins as inhibition of protein glycosylation by tunicamycin led to a decreased adhesion. Removal of serum from the assay, leaving cell glycoproteins as the only feeding source, increased adhesion. If fetuin, a glycoprotein deglycosylated by Cc5, was added to the serum-depleted medium, adhesion decreased. We conclude that adhesion of Cc5 to cells is linked to feeding through the Gpd complex. Further work will be conducted to find out whether adhesion could be important during infection.





**FEMS-2226**

**Bacterial pathogenicity**

**EXPRESSION OF RESUSCITATION PROMOTING FACTORS DURING IN VITRO GROWTH AND MACROPHAGE INFECTION**

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**Background**

Members of the *Mycobacterium tuberculosis* complex are the causative agents of tuberculosis, a major global health threat. The majority of individuals are thought to be infected latently whereby the bacteria are in a non-replicating dormant state, and the individual is asymptomatic. Many of the proteins secreted by this organism have been shown to be essential for the virulence, survival and dormancy, and function in a variety of different roles. The resuscitation promoting factors (Rpfs) are one such family of proteins whose peptidoglycan hydrolyzing activities have been correlated with the resuscitation of dormant bacteria; however, the precise molecular mechanisms underlying this process remain poorly understood.

**Objectives**

The aim of this work is to gain insight into the expression and localisation patterns of Rpfs during bacterial growth and macrophage infection.

**Methods**

We have generated a number of Rpf deletion mutants and Rpf specific polyclonal antibodies to monitor Rpf expression *in vitro* using flow cytometry, and during *Mycobacterium marinum* infection of murine macrophages by immunofluorescence and confocal microscopy.

**Conclusions**

Having observed Rpfs at the level of individual bacteria, we conclude that a subset of

the mycobacterial population express Rpf at a detectable level during *in vitro* growth, and the infection process.

**FEMS-1795**  
**Bacterial pathogenicity**

**DISTRIBUTION OF GENES RESPONSIBLE FOR VIRULENCE IN CRONOBACTER STRAINS**

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**Background**

*Cronobacter* spp. was proposed as a new genus in 2008. All the members of *Cronobacter* are considered to be potentially pathogenic; however recent epidemiological studies indicated differences in virulence. The most often isolated species from clinical samples were *C. sakazakii* and *C. malonaticus*. Some reports revealed the connection between sequence type and virulence of particular strain. It has been shown that sequence type 4 (ST4 – *C. sakazakii*) is prevalent for meningitides, ST7 (*C. malonaticus*) is often associated with adult infections. These sequence types are considered as the evidence of virulence.

**Objectives**

The objectives of this work were to implement PCR specific for virulent proteins and to reveal connection between sequence type (MLST) and virulence genes.

**Methods**

Several factors responsible for virulence were targeted: the system for iron acquisition, outer membrane protein A, haemolysin and metalloprotease placed on chromosome and type six secretion system, secretion system FHA and outer membrane protease placed on plasmids.

PCR protocols for the detection of corresponding genes were applied to all *Cronobacter* species and compared with determined sequence type.

**Conclusions**

The predominance of some virulence genes was observed within particular species, in some cases even with sequence type; for example, haemolysin was detected in all tested *C. sakazakii* strains and in *C. malonaticus* presenting sequence type 7.

This work was supported by the Czech Grant Agency (13-23509S).

**FEMS-3117**

**Bacterial pathogenicity**

## **OUTER MEMBRANE PROTEIN 1 P1 IS THE CEACAM-BINDING ADHESIN OF HAEMOPHILUS INFLUENZAE**

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### **Background**

*Haemophilus influenzae* (*Hinf*) is a Gram-negative pathogen colonizing the upper respiratory tract mucosa. *Hinf* belongs to a group of human-restricted bacteria, which bind to carcinoembryonic antigen related cell adhesion molecules (CEACAMs) on epithelial cells. Adhesion to CEACAMs is thought to be mediated by the *Hinf* outer membrane protein (OMP) P5 promoting establishment of the pathogens in the human nasopharynx.

### **Objectives**

Aiming at preventing *Hinf* colonization, we sought to identify the molecular requirements for *Hinf* binding to CEACAMs.

### **Methods**

Binding assays with soluble receptor ectodomains, followed by flow cytometric analysis or Western Blotting, were used to characterize CEACAM-binding profiles of wildtype and mutant *Hinf*. OMP P1, and not OMP P5, was identified as the CEACAM-binding adhesin. Multiple amino acid sequence alignment of P1 combined to heterologous expression of wild-type, chimeric, or mutated P1 in *E. coli* depicted the molecular details of the P1-CEACAM interaction.

### **Conclusions**

Surprisingly, *Hinf* P5 mutants still avidly bind CEACAMs and *Hinf* P5 expressed in *E. coli* fails to mediate CEACAM targeting. Instead, a genetic screen identifies *Hinf* P1 as the CEACAM-binding adhesin. Deletion of P1 in *Hinf* and heterologous expression in *E. coli* demonstrate that P1 is necessary and sufficient to bind several human CEACAMs. Concordantly, when expressed on the surface of *E. coli*, P1 promotes adhesion to and invasion into epithelial cells. Structure-activity relationship investigations with P1 mutants demonstrate that several flexible extracellular loops allow P1 to engage human CEACAMs. These results provide the first evidence for the involvement of the major outer membrane protein P1 of *Hinf* in pathogenesis.

**FEMS-2695**

**Bacterial pathogenicity**

## **LOCAL AND SYSTEMIC EFFECTS OF EXPERIMENTAL MURINE HELICOBACTER PYLORI INFECTION.**

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### **Background**

Despite its late-in-life pathogenicity (gastric cancer and peptic ulcer disease), due to its potential early-life benefits (protection against childhood-onset asthma, diarrheal disease, and reflux esophagitis), *Helicobacter pylori* (*Hp*) might be considered a commensal. The ongoing disappearance of *Hp* from the human microbiome is concerning as little is known about the influence of *Hp* on the host's microbiome and immunity.

### **Objectives**

In this study, we aimed to investigate the interactions of *Hp* with its host in a mouse model.

### **Methods**

We infected C57/Bl6 mice with *cagA*+ *Hp* PMSS1, or not (control). Groups of mice were serially sacrificed over a period of 6 months and host responses analyzed.

### **Conclusions**

All inoculated mice were *Hp*-positive when sacrificed, as verified with PCR and culture and displayed gastric histology typical for *Hp* colonization. All infected mice showed high levels of IgM/IgG to *Hp* but not to CagA. Nanostring® analysis, performed on stomach and lung, revealed a large number of immune genes in the stomach with increased expression. Immune gene expression was also altered in the lung and flow-cytometry confirmed significantly higher levels of Th17-cells in infected mice compared to controls. Extracted DNA from fecal pellets, gastric, ileal and cecal contents were subjected to 16S rRNA gene sequencing to determine changes in the microbiome due to *Hp* colonization. Overall, control and colonized mice displayed

different population structures in both their gastric and intestinal microbiota. These findings indicate that in this mouse model, *Hp* influences both the microbial population structure and local and distant host immune responses.

**FEMS-0918**  
**Bacterial pathogenicity**

**MIDDLE EAST ORIGINATED INFECTIOUS DISEASES THREATENING TURKEY  
:SIX TURKISH CASES INFECTED IN IRAQ WITH RARE SEROGROUPS OF  
LEGIONELLA PNEUMOPHILA**

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**Background**

Although *L.pneumophila* serogroup(sg)1 is the common disease causing serogroup, rare serogroups can also cause legionellosis.

**Objectives**

We aim to present six cases of legionellosis, of which four cases presented as Pontiac fever and the other two cases presented as Legionnaires' disease. And then emphasize the regional epidemic and pandemic threats of infectious diseases that are transmitted between neighboring countries.

**Methods**

We reported 6 cases of legionellosis caused by rare sg of *L. pneumophila*. Their diagnosis was supported with clinical, Radiological and serological findings and serogrouping *L. pneumophila*. The analyses of IgG and IgM were performed in our unit using the Anti-Legionella Pneumophila Indirect Immunofluoresan IgM, IgG kit (Euroimmun AG, Leubeck, Germany). All six serum samples sent, Euroimmun AG Clinical Immunology Laboratory in Lubeck, Germany for serogrouping.

**Conclusions**

Two of them were diagnosed with Legionnaires' disease caused by *L.pneumophila* sg12 (index case KY ) and sg11 (his wife ZY ) and 4 of the cases



were diagnosed with Pontiac fever caused by *L.pneumophila* sg14 (ZY), sg4(AK), sg4 with sg6 (BT) whereas the sg of *L.pneumophila* detected in ZC could not be identified. This study reemphasized that not only *L. pneumophila* serogroup 1, but other rare serogroups might cause also legionellosis which may increase in frequency and cause regional epidemics. It should be noted that these epidemics can also become a threat and a severe public health problem for all countries. Global-scale solutions should be developed for epidemics that could threaten this region

**FEMS-1651**

**Bacterial pathogenicity**

**THE PATTERNS OF EPIYA MOTIFS AMONG CAGA POSITIVE HELICOBACTER PYLORI STRAINS: A PROSPECTIVE STUDY IN TURKISH POPULATION WITH EURASIAN GEOGRAPHICAL FEATURES**

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**Background**

Geographical variation in the frequency of various gastroduodenal pathologies was shown to be related to the geographical diversity of H.pylori cagA/EPIYA patterns.

**Objectives**

We sought to determine the EPIYA patterns of cagA-positive H.pylori strains isolated from patients with different endoscopic diagnoses and from patients with gastric cancer (GC) who lived in Istanbul and surroundings areas, a Western region of Turkey located both in Europe and Asia.

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**Methods**

One hundred and fifty-eight cagA-positive strains were included in the study; 84 were isolated from patients with gastritis (G), 38 were from patients with GC, 22 were from patients with duodenal ulcers (DU) and 14 were from patients diagnosed endoscopically with having normal gastrointestinal systems (NGIS). Specific primers were used for the detection of EPIYA patterns and representative bands were also confirmed by DNA sequencing.

**Conclusions**

EPIYA A, B, C or D were found in 142 of the 158 strains (89.9%). EPIYA-ABC was detected in 83 H. pylori 50 (59.5%) were isolated from patients with G, 14 (63.7%) were isolated from patients with DU, 9 (23.7%) were isolated from patients with GC and 10 (71.4%) were isolated from patients with NGIS. EPIYA-C with  $\geq 2$  repeats was detected in 34 (21.5%) cagA-positive H.pylori cases and 22(64.7%) of these cases involved GC. The most common EPIYA pattern isolated from cases with different endoscopic diagnoses was the Western type EPIYA-C. EPIYA-C with  $\geq 2$  repeats was

found to be more common in cases with GC; in other cases with different endoscopic diagnoses, EPIYA-C with one repeat was the most frequent.

**FEMS-1485**

**Bacterial pathogenicity**

**IDENTIFICATION AND CHARACTERIZATION OF STRESS FIBER INDUCING EFFECTOR PROTEIN OF VIBRIO PARAHAEMOLYTICUS T3SS2**

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**Background**

*Vibrio parahaemolyticus* is an important pathogen that causes food-borne gastroenteritis in humans. The type III secretion system encoded on chromosome 2 (T3SS2) plays a critical role in the enterotoxic activity of this bacterium. Tissue culture analysis has shown that T3SS2 causes two dramatic changes in the actin cytoskeleton: the accumulation of F-actin beneath bacterial microcolonies and the induction of actin stress fibers. VopV, which is enterotoxic effector, exhibits F-actin binding activity and is responsible for the F-actin accumulation phenotype. However, the mechanisms that underlie T3SS2-dependent actin stress fiber formation and the main effector have not been elucidated.

**Objectives**

The aim of this study is to identify effector protein responsible for T3SS2-dependent stress fiber formation.

**Methods**

*V. parahaemolyticus* strain RIMD2210633 (KP-positive, serotype O3:K6) was used for parent strain. A four-primer polymerase chain reaction (PCR) technique was used to engineer an in-frame deletion mutation. Caco-2 cells were infected with *V. parahaemolyticus* for 3 h at a multiplicity of infection (MOI) of 10. Actin was detected with Alexa Fluor® 488-phalloidin. Cellular and bacterial DNAs were stained with Hoechst 33258.

**Conclusions**

After screening candidate ORFs encoded within the Vp-PAI region, a known pathogenicity island in pathogenic strains, we identified an effector candidate protein involved in stress fiber formation. A deletion of this gene did not affect to T3SS2-dependent enterotoxicity in rabbit ileal loop model, but this mutant caused a dramatic change in actin stress fiber formation, thereby suggesting that this protein is a T3SS2 effector involved in the induction of stress fiber formation.

**FEMS-1935**

**Bacterial pathogenicity**

## **STRUCTURE-FUNCTION ANALYSIS OF THE TYPE 3 SECRETION SYSTEM TRANSLOCATOR IPAB**

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### **Background**

The Type 3 Secretion System (T3SS) enables the invasion the human intestinal gut mucosa by *Shigella flexneri*. The T3SS consists of a membrane embedded basal body and an extracellular needle. At the distal end of the needle a tip complex senses and binds the host cell membrane. Upon binding secretion of effectors from the bacterial cytoplasm through the T3SS into the host cell is activated. The secretion of effectors follows a defined order and prior secretion unfolding of the effectors enables the transport through the needle. Early secreted effectors, known as translocators form pores in plasma membranes. The translocators such as the invasion plasmid antigen B (IpaB) are bound to chaperones (IpgC) in the bacterial cytoplasm. For successful infection the IpaB/IpgC complex disassembles and unfolded IpaB is secreted while IpgC remains in the bacterial cytoplasm. Thus, IpaB adopts different folding states before and after secretion which is studied by X-ray crystallography and other biophysical methods.

### **Objectives**

Analysis and comparison of different IpaB conformations with the aim to gain insight into IpaB function.

### **Methods**

Detergents for stabilizing the hydrophobic domains of full length IpaB were used for crystallization trials as well as full length IpaB in complex with IpgC without detergent. In another approach soluble fragments of IpaB were successfully crystallized and the structure determined. IpaB fragments were also cocrystallized with IpgC and the structural determination is in progress.

### **Conclusions**

Based on this study, future design of small inhibitors preventing correct IpaB folding could be beneficial for therapeutic-clinical use against *Shigella* infections.

Barison (2012) *FASEB26*

Senerovic (2012) *CellDeathDis3*

**FEMS-2083**

**Bacterial pathogenicity**

**MIXED INFECTION ENHANCES THE VIRULENCE OF AEROMONAS IN THE CAENORHABDITIS ELEGANS MODEL**

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**Background**

*Aeromonas* virulence remains poorly understood, virulence of any given strain being hardly predictable. In addition, *Aeromonas* human infections involve mixed *Aeromonas* isolates (5-10% cases) with an unknown impact on virulence.

**Objectives**

To evaluate virulence of strains recovered from mixed aeromonad infections, alone and in association.

**Methods**

Twelve *Aeromonas* isolates recovered in pairs from 6 mixed infections were tested against the *Caenorhabditis elegans* virulence model. Dead worms were counted each day during 15 days. Median time for killing worms (TD50) was determined for single isolates and for natural and experimental pairs. Experiments were repeated 3 times, independently. Genomes of 7 isolates were sequenced using an Illumina Myseq; virulence-associated genes were sought within the draft genomes.

**Conclusions**

Eight isolates were weak killers (TD50≥7 days) when they were administered alone, two pairs showed an enhanced virulence: the TD50 was significantly lowered from 7.8 and 8.3 days to 3.3 days (couple 76c+77c) and from 6 and 9 days to 4.5 days (couple 25a+25b). Synergy was also observed for 5 of the 14 experimental pairs tested, each including one strain from the natural synergistic couples. Only couples involving strains from distinct species showed synergy. The genome content of virulence-associated genes failed to explain virulence synergy and to identify pathotypes, although some virulence-associated genes present in some strains were absent from the companion strain (e.g., T3SS).

Synergistic virulence observed between infectious *Aeromonas* isolates stresses to consider *Aeromonas* infection process at the community level and not only according to virulence content as in pathotype concept.

**FEMS-2389**

**Bacterial pathogenicity**

**STAPHYLOCOCCUS AUREUS-DERIVED MEMBRANE VESICLES EXACERBATE SKIN INFLAMMATION IN ATOPIC DERMATITIS**

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**Background**

Skin colonization or infection with *Staphylococcus aureus* is known to trigger aggravation of atopic dermatitis (AD). However, the exact mechanisms by which *S. aureus* can worsen AD are unknown.

**Objectives**

We investigated whether and how *S. aureus*-derived membrane vesicles (MVs) contribute to worsening of AD.

**Methods**

HaCaT cells were treated with *S. aureus* MVs and were analyzed for the expression of pro-inflammatory cytokine genes. Immunopathology and cytokine gene profiles were analyzed after topical application of *S. aureus* MVs to AD-like skin lesions in a mouse model.

**Conclusions**

Intact MVs from *S. aureus* delivered their components to keratinocytes and stimulated pro-inflammatory cytokine gene expression. However, MVs with a disrupted membrane neither delivered their components to keratinocytes nor resulted in cytokine gene expression. A knockdown of nucleotide-binding oligomerization domain 2 by using small interfering RNAs completely suppressed IL-8 gene expression. Topical application of *S. aureus* MVs to AD-like skin lesions in the mouse model induced massive infiltration of inflammatory cells and the resulting eczematous dermatitis. This inflammatory reaction was associated with a mixed Th1/Th2 immune response and enhanced expression of chemokine genes in AD-like skin lesions. *S. aureus* MVs delivered effector molecules to host cells and triggered an inflammatory response both *in vitro* and *in vivo*. MVs produced by *S. aureus* colonizing or infecting AD skin lesions may be responsible for worsening of AD. Thus, *S. aureus* MVs are a new therapeutic target for the management of AD aggravation.





**FEMS-0905**

**Bacterial pathogenicity**

## **THE INTIMIN PERIPLASMIC DOMAIN MEDIATES DIMERISATION AND BINDING TO PEPTIDOGLYCAN**

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### **Background**

Intimin and Invasin are prototypical inverse (Type Ve) autotransporters and important virulence factors of enteropathogenic *Escherichia coli* and *Yersinia spp.* respectively. In addition to a C-terminal extracellular domain and a  $\beta$ -barrel transmembrane domain, both proteins also contain a short N-terminal periplasmic domain that, in Intimin, includes a lysin motif (LysM), which is thought to mediate binding to peptidoglycan.

### **Objectives**

We investigated the functions of the Intimin periplasmic domain, particularly to study its role in peptidoglycan binding and dimerisation.

### **Methods**

We used pull-down, solid phase binding and in vivo binding assays to demonstrate that the Intimin LysM binds to peptidoglycan. Dimerisation of the periplasmic domain was shown by analytical size exclusion chromatography and in vitro cross linking experiments. We further solved the structure of the Intimin LysM using NMR.

### **Conclusions**

We show that the periplasmic domain of Intimin does bind to peptidoglycan both in vitro and in vivo, but only under acidic conditions. We were able to determine a dissociation constant of 0.8  $\mu$ M for this interaction, whereas the Invasin periplasmic domain, which lacks a LysM, bound only weakly in vitro and failed to bind peptidoglycan in vivo. Furthermore, in contrast to previous reports, we show that the periplasmic domain mediates dimerisation.

We further show that dimerisation and peptidoglycan binding are general features of LysM-containing inverse autotransporters. Peptidoglycan binding by the periplasmic domain in the infection process may aid in resisting mechanical and chemical stress during transit through the gastrointestinal tract.

**FEMS-0900**

**Bacterial pathogenicity**

**BRINE SHRIMP: A HOST MODEL SYSTEM TO STUDY BACTERIAL VIRULENCE AND SYMBIOSIS**

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**Background**

Brine shrimp is an aquatic crustaceans belonging to a genus of *Artemia*. This organism is widely used for testing the toxicity of chemicals and for live food in the larviculture of economically important fishes and crustaceans.

**Objectives**

In this study, a brine shrimp, *Artemia salina* was evaluated for the bacterial infection and symbiotic host model.

**Methods**

Brine shrimp nauplii were incubated in petri dish containing 5 ml of autoclaved artificial seawater. Various numbers of bacterial cells were added to the seawater and incubated at 28°C for several days. The survival of shrimps was daily scored after the addition of the bacteria and the existence of bacteria in shrimp gut was observed by fluorescence microscope.

**Conclusions**

Pathogenic bacteria caused significant death of brine shrimps reflecting their virulence, but some shrimps surviving the infection were found to grow bigger and faster. Both *E. coli* and *P. aeruginosa* could survive in the brine shrimp gut, but *E. coli* was able to survive only for limited period whereas *P. aeruginosa* survived more and longer in the gut than *E. coli*. Furthermore, we found that the pre-infection of avirulent *P. aeruginosa* strain improves the survival of brine shrimp in the challenge of other pathogenic bacteria. These results strongly suggest that survival of *P. aeruginosa* as a symbiont has a beneficial effect on host and brine shrimps may be a valuable artificial model to study symbiosis.

**FEMS-3018**

**Bacterial pathogenicity**

**DIRECT INVOLVEMENT OF CYCLIC AMP (cAMP) AND cAMP RECEPTOR PROTEIN (CRP) IN NATURAL COMPETENCE REGULATOR TFOX EXPRESSION**

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**Background**

*Vibrios* are normal inhabitants in the estuarine and marine environments, where they exist both as free-living bacteria and in association with phytoplankton, zooplankton, and crustaceans. Chitin-induced competence found in several *Vibrios* represents a newly appreciated mode of horizontal gene transfer in these marine bacteria and undergoes tight regulation. Sxy (also called TfoX) and CRP are two important activator proteins in the competence development. Sxy functions synergistically with CRP to activate the competence genes regulon expression.

**Objectives**

Sxy/TfoX expression has been shown to be regulated by RNA second structure and sRNA *tfoR* at the post-transcriptional level. In this study, we investigated the transcriptional regulation of *tfoX* expression by cAMP-CRP complex.

**Methods**

qRT-PCR and complement assay were used to analyze the *tfoX* mRNA level. Promoter transcriptional fusion and site-directed mutagenesis of the putative CRP-binding sites were applied to determine the *tfoX* promoter activities. EMSA assay was employed to demonstrate the direct binding of CRP to the *tfoX* promoter. The transcriptional start site of *tfoX*<sup>VF</sup> was determined by 5'RACE.

**Conclusions**

CRP positively regulates the *tfoX* expression at the transcriptional level; there are two functional CRP binding-sites on *tfoX*<sup>VC</sup> promoter region and CRP directly binds to the promoter region to initiate its transcription; transcriptional start site of *tfoX*<sup>VF</sup> was mapped at -126 nucleotides upstream of translational start site; similar -10 motifs and putative CRP-binding sites on different species' *tfoX* promoter indicate CRP regulation of *tfoX* is a conserved regulatory mechanism in *Vibrio* species.

**FEMS-0944**

**Bacterial pathogenicity**

## **REGULATION OF THE CYCLIC AMP SIGNALING PATHWAY OF KLEBSIELLA PNEUMONIAE IN TYPE 3 FIMBRIAE EXPRESSION**

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### **Background**

*Klebsiella pneumoniae* (Kp) is the predominant pathogen isolated from liver abscesses of diabetic patients in Asian countries. Although elevated blood glucose levels cause various immune problems, its effects on Kp virulence remains largely unknown. In bacteria, cyclic AMP (cAMP), a well-known second messenger, plays a fundamental role in global gene regulation in response to exogenous glucose levels. However, targets regulated by cAMP signaling pathway remain uncharacterized in Kp.

### **Objectives**

To investigate the role of cAMP signaling pathway in Kp pathogenesis.

### **Methods**

DNA microarray, promoter activity assay, qRT-PCR, western blotting assay, biofilm formation, electrophoresis mobility shift assay

### **Conclusions**

Based on the analysis of DNA microarray results, we noted a serial of type 3 fimbrial genes was apparently increased in  $\Delta crp$  strain. It indicates that CRP could repress the type 3 fimbriae expression. By using promoter activity assay, western blotting, and qRT-PCR to confirm that the MrkA expression, the major pilin of type 3 fimbriae, was regulated by cAMP signaling pathway. However, no typical CRP binding site was found in the sequence of  $P_{mrkA}$ , suggesting CRP regulates the *mrkA* transcription via other mediator(s). According to previous studies, multiple proteins, which include Fur, MrkH, MrkI, MrkJ, and YjcC have been shown to mediate the type 3 fimbriae expression. The underlying mechanism of cAMP signaling pathway and the mediators as described above in regulating the type 3 fimbriae expression was investigated in this study.

**FEMS-1724**

**Bacterial pathogenicity**

## **IDENTIFICATION OF FITNESS DETERMINANTS IN PSEUDOMONAS AERUGINOSA INFECTION**

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### **Background**

*Pseudomonas aeruginosa* is a clinically highly relevant opportunistic pathogen known to cause acute and chronic infections. This bacterium can adapt to the host by environmentally driven alterations in gene expression as well as genomic alterations in response to selective pressures by the environment.

### **Objectives**

The aim of this study is to identify genetic adaptations that are key to fitness and successful establishment of acute and chronic infections by *P. aeruginosa*.

### **Methods**

We have developed an *in vivo* model for *P. aeruginosa* biofilm formation in CT26 tumor-bearing mice. This murine model offers the unique opportunity to screen the contribution of individual genes to the fitness of *P. aeruginosa* during *in vivo* biofilm formation. Furthermore, the inclusion of an acute lung infection model allows the comparison of fitness parameters in chronic to acute infections. These parameters will be investigated using (i) a PA14 transposon mutant library (Skurnik *et al.* 2013) as forward genetic approach and (ii) a reverse genetic approach by passaging bacteria in the animal host. To evaluate the contribution of individual genes onto bacterial fitness, isolated bacteria are quantified using high-throughput insertion and whole-genome sequencing, respectively.

All animal experiments were approved by the local regulatory board LAVES (Niedersächsisches Landesamt für Verbraucherschutz und Lebensmittelsicherheit) and the EU Directive 86/609/EEC, OJ L 358, p 1-28; 18.12.1986.

### **Conclusions**

Here we present a dual approach to gain insights into fitness determinants important during *P. aeruginosa* infection in different host environments.

**FEMS-0780**

**Bacterial pathogenicity**

**WILD-TYPE AND NONBINDING MUTANT OF TRANSFERRING BINDING PROTEIN B (TBPB) HAEMOPHILUS PARASUIS SEROVAR 5: IMMUNOENZYMATIC AND GENE EXPRESSION STUDIES**

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**Background**

Glasser's disease, caused by *Haemophilus parasuis* (*Hps*), produces annually pig significant economic losses. It is broadly known the relevance of transferrin binding proteins (Tbps) as virulence factor for developing this disease in the host

**Objectives**

Subunit vaccines based on the native, wild-type TbpB and on a mutant TbpB (TbpBY167) (losing its ability for binding porcine transferrin) from *Hps* serovar 5 were designed

**Methods**

Immunization and challenge (with an intratracheal administration of 10<sup>8</sup> CFU/ml of the Nagasaki strain of *Hps* 5) experiment was implemented in colostrum deprived pigs for comparing the protective response developed for wild-type and mutant proteins. Clinical symptoms were monitored after challenge and pathological findings were determined upon necropsy. All the pigs immunized with adjuvant alone and challenged did not survive more than 72 hours. On the other hand, most of the pigs immunized with wild-type TbpB survived the full two weeks after challenge but clinical signs and severe chronic lesions were observed. All the pigs immunized with the mutant also survived until the end of the study, without signs of infection.

Were confirmed the results by indirect ELISA test, by immunohistochemistry and real-time quantitative PCR was used to determine the relative expression of 27 immune-related genes in the lungs of the pigs belonging to experimental groups, at the necropsy time

**Conclusions**

The highest protective response was that afforded by the mutant.

Genes associated with the inflammation are highly overexpressed in the group of only infected animals and this does not happen in the other two experimental groups.

**FEMS-1734**

**Bacterial pathogenicity**

## **GENETIC DIVERSITY OF COLICIN E1 AND FIMBRIAE TYPE I IN ESCHERICHIA COLI STRAINS**

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### **Background**

Uropathogenic *E. coli* (UPEC) strains are causative agents in the majority of human urinary tract infections (UTIs). UPEC strains contain several virulence factors allowing them to colonize human urinary tract (e.g. fimbriae type I). Moreover, higher prevalence of colicin E1 like plasmids and a possible role of colicin E1 protein as a potential important virulence factor of UPEC strains was described in UPEC strains.

### **Objectives**

The main aim of the study focused on the genetic variability of colicin E1 and *fimH* gene (encoding the mannose-sensitive FimH adhesin of fimbriae type I) between human intestinal and extraintestinal *E. coli* strains (UPEC strain).

### **Methods**

In the set of 58 human fecal *E. coli* strains and 55 UPEC strains, *fimH* gene and colicin E1 gene was sequenced and analyzed. All clinical samples were collected after patients gave informed consent. The study was approved by the ethics committee of the Faculty of Medicine, Masaryk University, Brno, CZ.

### **Conclusions**

Two different colicin E1 genetic variants were described. Colicin E1-A genetic variant that prevailed among human fecal *E. coli* had 1566 bp and colicin E1-B genetic variant (prevailed in UPEC strains) had 1569 bp. The main genetic difference identified between these variants encoded colicin receptor domain. A significant increase of the substitution of residue Arg166 - His166 in fimbriae-associated pilin domain of FimH was identified in UPEC strains. It is possible that genetic variability identified in genes encoding colicin E1 and FimH modify virulence of UPEC strains.

FEMS-2996

Bacterial pathogenicity

## DIFFERENCES AND SIMILARITIES BETWEEN *S. ENTERICA* SEROVARS TYPHIMURIUM AND ENTERITIDIS FIS MUTANTS UNDER IN VITRO CONDITIONS

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### Background

*Salmonella enterica* is the causal agent of a widespread and neglected infectious disease in the world, that despite efforts, it lacks completely effective vaccine formulations. The non-typhoidal serovars Typhimurium and Enteritidis are frequently responsible for salmonellosis outbreaks, usually associated with gastrointestinal infections, but they can spread systemically, particularly in immunocompromised patients or those who suffering from malaria. In Africa, non-Typhi *Salmonella* comprises around 50% of reported cases of bacteremia and are associated with nearly 20% of the fatal cases of salmonellosis. It's crucial to test the potential of new mutants and new serovars for use as live attenuated vaccines to contain the disease.

### Objectives

We used recent clinical isolates of *S. Typhimurium* and *S. Enteritidis* to develop the strains LGBM01 $\Delta$ *fis* and SEnPT4 $\Delta$ *fis*, respectively, and evaluated their phenotype under *in vitro* conditions.

### Methods

The phenotypic tests performed included analysis of growth in Luria Broth with measurements taken every hour, motility test on semi-solid agar plates and invasiveness in primary macrophages obtained from BALB/c mice.

### Conclusions

The results showed no significant differences in growth rate in Luria Broth between the mutant strains and the respective wild strain, but the motility in semi-solid agar plates was significantly decreased, at least 50%, in the mutant strains. Interestingly, the ability to invade primary macrophage cells was significantly affected only in the Typhimurium mutant strain. Those results showed an important difference for the role of the same DNA binding protein in these serovars that needs to be considered for future use in vaccine development.



**FEMS-1971**

**Bacterial pathogenicity**

## **PATHOGENIC FEATURES OF BACTERIAL STRAINS ISOLATED FROM SURGICAL RINSE WATER AND MEDICAL INSTRUMENTS**

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### **Background**

One of the potential sources for bacterial contamination in hospital environments is represented by the inadequate manual cleaning of medical instruments.

### **Objectives**

The purpose of this study was to investigate the pathogenic features of microbial strains isolated from sterile water use for rinsing the surgeons hands and surgical medical instruments, in order to establish potential contamination risks for patients.

### **Methods**

A number of 200 water samples and 400 surgical instruments have been processed for routine microbiological analyses, following the existent ISO guidelines. The isolated strains have been identified using miniAPI galleries and investigated for antibiotic susceptibility profiles and for the production of cell-associated (adherence to inert and cellular substratum, biofilm development) and soluble, enzymatic (hemolysins, lecithinase, lipase, caseinase, gelatinase, amylase, esculin hydrolysis, DN-ase) virulence factors, using phenotypic (disk diffusion method, selective media for enzymatic factors production) and PCR-based methods.

### **Conclusions**

Bacteria were isolated in 10% of the analyzed water samples and from 5% of the investigated instruments. The following bacteria were isolated in the frequency decreasing order: *Pseudomonas aeruginosa*, *Brevundimonas* sp., *Micrococcus luteus*, *Staphylococcus haemolyticus*, *S. warneri*, *P. putida*, *P. stutzeri*, *Stenotrophomonas maltophilia*, *Moraxella* sp. Pore-forming toxins and enzymes have been the most frequently produced, followed by caseinase and esculin hydrolysis. The genetic support of these virulence factors has been confirmed by PCR. The analyzed strains exhibited only natural resistance profiles, demonstrating the

environmental origin of the isolated strains. The highest frequency of pseudomonades suggests that biofilm formation within piping is the source of contamination of surgical rinse water.

**FEMS-0734**

**Bacterial pathogenicity**

**UNUSUAL INCREASE OF TYPHOID FEVER CASES IN JAPAN AND  
MOLECULAR EPIDEMIOLOGICAL ANALYSIS OF THE CAUSATIVE AGENT,  
SALMONELLA TYPHI**

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**Background**

Typhoid fever is a systemic infection caused by *Salmonella enterica* serovar Typhi and transmitted from human to human via food or drinking water; therefore, hygiene and sanitary conditions mainly determine its spread. As a result of improved public sanitation, most of the cases in Japan have been sporadic and have come from abroad in recent years. In 2013, however, we observed an unusual increase of typhoid fever cases which seemed to be domestic infection.

**Objectives**

In this study, we assessed increase of the typhoid fever patients without histories traveling abroad and examined molecular epidemiological analysis of *S. Typhi*.

**Methods**

A total of 27 typhoid fever cases without histories traveling abroad were identified in 2013 and 26 *S. Typhi* isolates were collected. All isolates were examined with phage type and MLVA.

**Conclusions**

Of the 26 isolates, 15 strains were classified as phage type A or B1 and formed major cluster with 5 types of single locus variants by cluster analysis of the MLVA profiles. It seemed that closely related strains were causative agents of increased typhoid fever cases in Japan, 2013. These 15 patients were reported during 5 months (July to November) peaked at September. They ranged in age from 6 to 83 years (median age 32 years) and the male:female ratio was 1:1.5. Interviews by physicians or public health officers could not reveal specific information which leads to epidemiological link; therefore we have no identifiable source of infection.

**FEMS-1708**

**Bacterial pathogenicity**

**COMPARATIVE TRANSCRIPTOMICS OF BURKHOLDERIA CONTAMINANS ISOLATES FROM DIFFERENT STAGES OF INFECTION IN CYSTIC FIBROSIS PATIENT**

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**Background**

*Burkholderia cepacia* complex (Bcc) bacteria pose major threat to cystic fibrosis (CF) patients. Bcc infections can result in untreatable and fatal cepacia syndrome (CS) which is manifested as rapid deterioration of lung functions and septicaemia. The mechanisms behind transition from Bcc chronic infection to CS remain unknown.

**Objectives**

To gain insight into processes involved in development of CS, we compared transcriptomes of a lung isolate (chronic infection) and a bloodstream isolate (CS) from the same patient. Both isolates were of the same sequence type (ST872) and belonged to the Bcc species *B. contaminans* which is predominant among CF infections in Argentina.

**Methods**

Both strains were cultivated in three growth media: sputum (natural habitat of lung isolate), heat-inactivated human serum (natural habitat of bloodstream isolate) and control mineral medium, each in biological triplicates. RNA was extracted in mid-log growth phase, sequenced using RNA-Seq technology (Illumina) and converted to normalized transcript level values.

**Conclusions**

The expression of approximately 1,300 (18%) genes differed more than 3-fold between lung and sputum isolate. The bloodstream isolate showed markedly increased expression of quorum sensing-regulated pathogenicity determinants (motility, extracellular proteases, AidA, lectins, Flp pilus), hypoxia-activated genes and two antifungal compound synthetic clusters (occidiofungin and pyrrolnitrin). Agar plate assays confirmed rapidly increased motility and proteolytic, hemolytic and antifungal activities of bloodstream isolate. Assessing these phenotypes might help to monitor the progress of infections by *B. contaminans*.

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**FEMS-2824**

**Bacterial pathogenicity**

## **CORRELATION BETWEEN *H. PYLORI* SPECIFIC VIRULENCE FACTORS AND HOST IMMUNE RESPONSE ASSOCIATED WITH CLINICAL OUTCOMES OF THE INFECTION**

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### **Background**

*H. pylori* is the main cause of chronic gastritis, ulcer and gastric cancer. The balance between the bacterial virulence factors and the host immune response in the infection determines the different clinical outcomes. The association between Th1, Th17, Treg cells and *H. pylori* infection has been identified, but the effect of the nine major *H. pylori* specific virulence factors; *cag A*, *vacA*, *oipA*, *babA*, *hpaA*, *napA*, *dupA*, *ureA*, *ureB* on Th1, Th17 and Treg cells response in *H. pylori* infected patients has not been fully elucidated.

### **Objectives**

To investigate the correlation of the *H. pylori* virulence factors with the manifestations of gastric disease by developing a multiplex-PCR and the relationship between these virulence factors with Th1, Th17 and Treg cells.

### **Methods**

Multiplex and qRT-PCR were carried out to detect the *H.pylori* specific virulence factors and relationship between these virulence factors with T cells.

### **Conclusions**

The multiplex-PCR was developed to rapid characterisation of nine *H. pylori* virulence genes with in a three PCR reactions. Due to the multiplex-PCR results there was no significant difference in prevalence of virulence factors in patients with gastritis and ulcer. However, prevalence of *napA* virulence factor was significantly higher in

patients with ulcer than gastritis. A positive correlation between the *dupA* virulence factor and IFN- $\gamma$  was observed in gastritis patients. Additionally, IL-17 expression was found significantly positively correlated with the *babA* virulence factor in ulcer patients. Furthermore, a novel expert derived model is developed to identify set of factors and rules distinguishing the ulcer patients from gastritis patients.

**FEMS-1995**

**Bacterial pathogenicity**

## **IMPACT AND ROLE OF THE RNA CHAPERONE HFQ IN VIRULENCE GENE REGULATION OF LEGIONELLA PNEUMOPHILA**

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### **Background**

*Legionella pneumophila*, the causative agent of the pneumonia-like Legionnaires' disease, is commonly found in aquatic habitats worldwide where it multiplies within protozoa. To adapt between intra- and extracellular environments, *L. pneumophila* evolved a biphasic lifecycle wherein it alternates between a replicative (non-virulent) and a transmissive (virulent) phase. This switch is governed by a complex regulatory network. Expression of the Host Factor Q protein (Hfq), a hexameric, RNA-binding protein and chaperon of small RNAs (sRNA) is life cycle regulated and implicated in virulence. How Hfq expression is regulated in *L. pneumophila* is not known.

### **Objectives**

Our aim was to understand how the growth-phase dependent expression of Hfq is regulated and whether a newly identified sRNA is implicated

### **Methods**

By Transcriptional Starting Site mapping of the *L. pneumophila* genome, a sRNA that is transcribed antisense to the *hfq* gene and is overlapping its 5'UTR region was identified. Thus, we postulate that this antisense RNA, named *anti-hfq*, might regulate the life cycle dependent expression of Hfq. We constructed and characterized the *L. pneumophila hfq* deletion mutant and *anti-hfq* overexpressing strains *in vitro* and *in vivo*.

### **Conclusions**

Our results show that Hfq expression is growth-phase dependently regulated by this newly identified antisense RNA. Infection assays revealed that Hfq is a virulence factor necessary for efficient replication in amoeba. Overexpression of *anti-hfq* led to a similar virulence phenotype as *hfq* deletion. This suggests an important role of Hfq and *anti-hfq* in virulence and in the regulatory network governing the biphasic life cycle of *L. pneumophila*.



**FEMS-1730**

**Bacterial pathogenicity**

**ISOLATION AND CHARACTERIZATION OF OUTER MEMBRANE VESICLES OF THE FISH PATHOGEN *PISCIRICKETTSIA SALMONIS*.**

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**Background**

*Piscirickettsia salmonis* is a Gram-negative intracellular bacterium that causes Piscirickettsiosis in salmonids farms in Chile. It was recently reported that *P. salmonis* produces exotoxins that play a role in the pathogenesis. However a delivery system has not yet been identified. Outer membrane vesicles (OMVs) are 10 to 300 nm spherical-bilayer structures discharged from the surface of many Gram-negative bacteria which are able to deliver toxins and virulence factors. However, the production of OMVs by *P. salmonis* has not been described.

**Objectives**

The aim of this study was to investigate if *P. salmonis* is able to produce OMVs and to realize a microscopic characterization of these vesicles.

**Methods**

*P. salmonis* was grown in basal broth supplemented with Cysteine (3.18 mM) and ferric chloride (0.05 mM) at 18°C until early stationary phase. Bacteria were removed by centrifugation (5000 x g, 10 min at 4°C) and the supernatant were filtrated through a 0.22-µm-pore-size filter. Then, vesicles present in the bacterial free supernatant were isolated by ultracentrifugation (125,000 x g, 2h at 4°C) and analyzed by SDS-PAGE. Finally, CHSE-214 cells infected with *P. salmonis* and negative stained OMVs were visualized by TEM.

**Conclusions**

*P. salmonis* is able to produce OMVs. The purified OMVs appeared as spherical vesicles of different sizes between 27.3 and 145.5 nm. SDS-PAGE analysis showed similar protein profile between OMVs and outer membrane extracts. Finally, OMVs were found into *P. salmonis*-containing vacuoles in infected CHSE-214 cells, suggesting that OMVs may contribute to the pathogenesis of *P. salmonis*.

**FEMS-2904**

**Bacterial pathogenicity**

**MULTIPLE COLONIZATION OF *HELICOBACTER PYLORI* IN PATIENTS WITH CHRONIC GASTRITIS OF REGIONS OF HIGH AND LOW RISK OF GASTRIC CANCER IN COLOMBIA**

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**Background**

Infection with virulent strains of *Helicobacter pylori* is necessary but not enough for the development of gastric cancer (GC) and its precursor lesions. In Nariño-Colombia, there are populations with high prevalence of *H. pylori* infection, but different GC risk. These results suggest different genetic characteristics of the circulating strains with varying degrees of pathogenicity.

**Objectives**

To evaluate multiple colonization of *H. pylori* isolated from patients with chronic gastritis from two populations with contrast in the risk of developing CG: Tumaco, low risk; Túquerres, high risk. Our hypothesis: there is a greater probability of multiple colonization of *H. pylori* in the population at highest risk for GC.

**Methods**

409 adult patients with dyspeptic symptoms were studied, of which gastric mucosa fragments from the antrum and body were used for the histopathological diagnosis, culture and genotyping (*cagA*, *vacA* and PCR-RAPD). 72 individuals were included, in whom *H. pylori* isolates was achieved in the three biopsies of gastric mucosa, (41/203) from Tumaco and (31/206) Tuquerres. Genetic similarity was calculated using the Nei's coefficient.

**Conclusions**

Genetic diversity was higher among isolates from Tuquerres (0.13) than Tumaco (0.07). After adjusting for age, sex and diagnosis, multiple colonization was 1.7 times more frequent in Tuquerres than Tumaco,  $p = 0.05$ . In Túquerres: high risk of gastric cancer, there was more likely to have multiple colonization. Based on the analysis of the results of the PCR - RAPD, we found greater genetic similarity from isolates of *H. pylori* in the population with low risk for developing GC

## **FEMS-1079**

### **Bacterial pathogenicity**

#### **IDENTIFICATION OF GENES INVOLVED IN VIRULENCE OF PUM505 PLASMID FROM PSEUDOMONAS AERUGINOSA**

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#### **Background**

pUM505 is a conjugative plasmid of 123 kilobases, originally isolated from a clinical strain of *Pseudomonas aeruginosa*. pUM505 possesses a pathogenicity island (PAI) of 78 genes, 64 of them have been found in the chromosomal PAPI-1 and PAPI-2 PAIs of *P. aeruginosa* PA14, a virulent clinical isolate, however it is unknown whether pUM505 is involved in virulence.

#### **Objectives**

The aim of this study is to determine if the plasmid increases the virulence of the *P. aeruginosa* PAO1 standard strain and to identify the genes responsible for this property.

#### **Methods**

Using as models of virulence lettuce leaves and *Dictyostelium discoideum* cultures pUM505 showed increased virulence of the PAO1 strain. To identify genes involved in virulence a gene bank was constructed by digesting the pUM505 plasmid and ligating the fragments to the vector pUCP20. A library of 120 clones was obtained in the Top10 strain of *E. coli* and PAO1 of *P. aeruginosa*. Four clones of this library were found to increase virulence in both *E. coli* and *P. aeruginosa*. The cloned inserts of these transformants was sequenced, and several ORF's of pUM505 were identified. The ORFs 2, 17 and 42 (*hop*) have homologues that have been reported involved in virulence.

#### **Conclusions**

In conclusion, pUM505 plasmid increases the virulence of its hosts due to some ORF's outside the pathogenicity island. The ORF's identified are currently under study.

**FEMS-1918**  
**Bacterial pathogenicity**

**ANALYSIS OF THE SALMONELLA TYPHIMURIUM SPI-1 TYPE 3 SECRETION  
SYSTEM SORTING PLATFORM**

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**Background**

Many Gram-negative bacterial pathogens use a type III secretion system (T3SS) to deliver virulence factors into the cytosol of host cells in order to establish and maintain an infection. This system relies on the sequential secretion of first structural and later effector proteins for its correct assembly and function. The hierarchy of substrate secretion has been proposed to be ensured by a cytoplasmic protein complex, called the "sorting platform".

**Objectives**

We aimed to investigate and characterize the Salmonella Typhimurium "sorting platform" and its components, particularly SpaO, with regard to both their structure and function.

**Methods**

The two expression products of the *spaO* gene were analyzed and the gene mutated to investigate the mechanism by which the second, shorter product is generated. The two proteins were analyzed by SDS-PAGE, mass-spectrometry, size-exclusion chromatography and multi-angle light scattering. The SpaO protein was further co-purified with other "sorting platform" components and the ability of the complex to bind T3SS substrates tested by surface plasmon resonance.

**Conclusions**

The gene for the component SpaO gives rise to both the full-length protein (SpaO) and a shorter product SpaO'. SpaO' is produced from an internal translation initiation site and is required for the stability of full-length SpaO. Together these two proteins associate with other "sorting platform" components to form a complex that is capable of binding different T3SS substrates.

**References**

Barison, N. et al., *Cell. Microb.* 15: 1809-1817 (2013)  
Lara-Tejero, M. et al., *Science* 331: 1188–1191 (2011)

**FEMS-3119**

**Bacterial pathogenicity**

**EFFECTIVE ROLE OF HELICOBACTER PYLORI INFECTION IN CANCER AND METASTASIS OF BONE MARROW DERIVED MESENCHYMAL STEM CELLS– AN INVITRO STUDY**

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**Background**

Recent studies have been shown that Helicobacter pylori has an effective role in the migration of bone marrow derived mesenchymal stem cells (BMD-MSCs) throw to gastric tissue .

**Objectives**

This study aimed to find the effect of H.pylori on as bacterial microenvironment on BMD-MSCs transforming into cancer stem cells and metastasis of the tumor cells .

**Methods**

BMD-MSCs were followed for evaluation by flow cytometric analysis with the hAbs for positive and negative of surface markers and treated under the osteogenic and adipogenic differentiation medium. BMD-MSCs were co-cultured with H. pylori and Gastric epithelial cell line (AGS). The expressions of MMP-2, MMP-9, p53 and bcl2 were examined by qRT-PCR

**Conclusions**

When mesenchymal stem cells are attracted to chronic H.pylori infection tissue and gastric ulcer for their tissue healing function, they will be trapped under special microenvironment. This study demonstrated that H. pylori increased the anti-apoptosis factor bcl-2 to keep BMD-MSCs alive and lead them to cancer stem cells by changing the regulation of p53. H. pylori increased the metastatic proteins MMP2 and MMP9 that shows the role of H.pylori on metastasis action. Focusing on H.pylori-induced molecular pathogenesis and the impact of microenvironment in gastric progenitor cells or BMD-MSCs will be crucial to identify the molecular targets in tumor initiation and the origin of gastric cancer.





**FEMS-1027**

**Bacterial pathogenicity**

## **A NOVEL, UNSUSPECTED FEATURE OF SALMONELLA ENTERICA SPI-1 BISTABILITY**

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### **Background**

*Salmonella enterica* pathogenicity island 1 (SPI-1) is a gene cluster that encodes a type 3 secretion apparatus and effectors involved in invasion of epithelial cells. A well known trait of SPI-1 is bistable expression, which generates SPI-1 ON and SPI-1 OFF subpopulations. The biological significance of SPI-1 bistability has been addressed by previous, insightful studies. Bistability has been viewed, for instance, as a division of labour involving self-destructive altruism by the SPI-1 OFF subpopulation (Ackermann et al. *Nature* 454, 987-90, 2008). Another study, however, has envisaged that the SPI-1 OFF subpopulation might benefit from inflammation triggered by the SPI-1 subpopulation (Stecher et al. *PLOS Biology* 5:2177-89, 2007). Furthermore, enhanced tolerance to antibiotics has been detected in slow-growing SPI-1 ON cells (Arnoldini et al. *PLOS Biology* e1001928, 2014).

### **Objectives**

In this communication we describe an additional, unsuspected feature of SPI-1 bistability.

### **Methods**

Single-cell analyses, such as flow cytometry and cell sorting were used to study the expression of SPI-1 in *Salmonella enterica* and its ability to invade epithelial cells.

### **Conclusions**

We show that a pure SPI-1 ON population obtained by bacterial cell sorting is non invasive, suggesting that the SPI-1 OFF subpopulation plays an active role in invasion. In support of this view, we also show that the invasion defect associated to unimodal expression of SPI-1 can be suppressed by mutations that permit formation of a SPI-1 OFF subpopulation.

**FEMS-2917**

**Bacterial pathogenicity**

**THE INVOLVEMENT OF STREPTOCOCCUS ANGINOSUS INFECTION AND THE ABERRANT ACTIVATION-INDUCED CYTIDINE DEAMINASE EXPRESSION IN HUMAN ORAL SQUAMOUS CELL CARCINOMA**

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**Background**

*Streptococcus anginosus* infection could be closely related with oral squamous cell carcinoma, although the mechanism underlying carcinogenesis is still unknown. Recent studies suggested that the aberrant expression of activation-induced cytidine deaminase (AID) in the epithelial cells could result in the generation of nucleotide alterations in tumor-related genes and possible malignant transformation of the AID-expressing cells.

**Objectives**

Using surgical tissue samples and cultured epithelial cells, a possible involvement of the aberrant AID expression in oral squamous cell carcinoma and *S. anginosus* infection was assessed.

**Methods**

The tissue specimens were obtained from the 17 patients with oral cancer after giving informed consent. *S. anginosus* infection and the aberrant AID expression were assessed by the species-specific PCR and RT-qPCR, respectively. Further, the aberrant AID expression and NF- $\kappa$ B activation were examined by a dual luciferase assay and RT-qPCR in three epithelial cell lines and the primary human gingival epithelial cells after stimulation with an *S. anginosus* antigen, SAA.

**Conclusions**

Both *S. anginosus* infection and the aberrant AID expressions were frequently observed in the tissue specimens (47% and 41%, respectively), and the infection was significantly correlated with the aberrant AID expression. The stimulation of the cultured cells with SAA could induce the NF- $\kappa$ B activation and aberrant AID expression in all the epithelial cells tested, and the addition of an inhibitor of NF- $\kappa$ B activation abrogated the aberrant AID expression. Thus, *S. anginosus* infection could be closely related with oral squamous cell carcinoma through the induction of the aberrant AID expression by *S. anginosus* antigen(s).

**FEMS-1975**

**Bacterial pathogenicity**

**IDENTIFICATION OF VIRULENCE FACTORS OF CRONOBACTER SPP.**

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**Background**

*Cronobacter spp.* are Gram-negative, motile, non-sporeforming, facultative anaerobic bacteria that have been implicated in rare but severe cases of illnesses predominantly in premature and newborn infants. These organisms have been isolated from a wide range of environments, including humans, different animals as well as (raw and processed) food and food production facilities.

**Objectives**

Although progress has been made during the last years, there is still a lack of knowledge on the virulence-associated factors and processes involved during pathogenesis.

**Methods**

To survey the pathogenesis of *Cronobacter*, a *C. sakazakii* ES5 transposon mutant library was screened in several cell lines for genes that are involved in to adhesion to and invasion into host cells as well as subsequent intracellular survival.

**Conclusions**

The majority of attenuated mutants carried insertions in genes involved in energy production and conservation, cell membrane biogenesis, biofilm formation, motility and metabolism. Several selected genes are being functionally analyzed such as genes for flagellar biosynthesis. Preliminary analyses showed that *C. sakazakii* flagella are necessary for adherence and efficient invasion of host cells. Using a specific monoclonal antibody against a living strain of *C. turicensis* 3032, flagellar motility could be inhibited and the ability to invade Caco-2 cells was reduced. Therefore, we present evidence that *Cronobacter* flagella play a crucial role in colonization of host cells.

**FEMS-1138**

**Bacterial pathogenicity**

**TRANSCRIPTOME ANALYSES TO UNDERSTAND INFECTION MECHANISM OF BURKHOLDERIA GLUMAE IN TWO DIFFERENT INFECTION SITES OF RICE**

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**Background**

*Burkholderia glumae* is a causal agent in grain and sheath rot, leading to severe damages in many rice-growing countries under the favorable conditions such as high temperature and humidity. However, its molecular mechanisms are not yet fully understood. For understating of infection mechanism, we analyzed *B. glumae* transcriptome from different infected rice tissues.

**Objectives**

We investigated molecular and physiological changes of *B. glumae* through comparative analyses of differentially expressed genes (DEGs) between in infected grains and in stems.

**Methods**

To gain a genome-wide gene expression profiling, we compared *B. glumae* transcriptome in stem and grain infection sites using the RNA sequencing. We then performed KEGG pathway and module enrichment of differentially expressed genes.

**Conclusions**

Most enriched metabolic and signaling pathways of *B. glumae* in infected stem tissues were bacterial chemotaxis-mediated motility, ascorbate metabolism, and sugar transporters including arabinose and xylose. However, we have confirmed different expression levels of genes involved in those pathways from the infected grains. For example, genes involved in flagellar assembly pathway were strongly down-regulated compared to those of stem tissues. Starch metabolisms were found to be highly enriched in infected grain tissues. Our study provides *in vivo* transcriptional profiling of *B. glumae* in two different tissues. Additionally, comparative analysis of *B. glumae* transcriptome obtained from the stem and grain will give clues on common and unique infection mechanisms in different tissues.

**FEMS-2055**

**Bacterial pathogenicity**

**PREVALENCE OF COLIBACTIN GENES IN ESCHERICHIA COLI RECOVERED FROM URINARY TRACT INFECTION FROM PATIENT WITH GYNECOLOGICAL CANCER**

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**Background**

Patients with gynecological cancer have increased risk for urinary tract infection (UTI) in which *Escherichia coli* is the main bacteria. The rates of antimicrobial resistance are increasing among these bacteria. Moreover, *E. coli* exhibits multiple accessory traits, including yersiniabactin. This virulence factor is associated with a peptide-polyketide compound, colibactin. This genotoxin, which damages DNA in eukaryotic cells, is related to the formation of colorectal cancer. However, little is known about colibactin production ratio in *E. coli* and its relation with antimicrobial resistance.

**Objectives**

To assess the prevalence of yersiniabactin and colibactin genes in a culture collection of *E. coli* isolated from patients with gynecological cancer with UTI and its relation with antibiotic resistance profile.

**Methods**

Two hundred eighteen *E. coli* UTI isolates were analyzed by PCR for detecting the phylogenetic group, *fyuA*, *clbB* and *clbN* genes. The isolate's antibiotic resistance profile was performed previously. Statistical analysis was performed using Fisher's test.

**Conclusions**

Virulence genes *clbB* and *clbN* were detected in 14.7% (32/218) of *E. coli* isolates. All strains in which these genes were detected belonged to the phylogenetic group B2 and had *fyuA* gene. Interestingly, multidrug resistance, defined as resistance to three or more antibiotic classes, showed to be a negative factor for the presence of colibactin production-related genes ( $p=0.0008$ ). Colibactin genes were not detected in aminoglycosides, gentamicin and tobramycin, resistant strains ( $p=0.05$ ) neither in fluoroquinolones, ciprofloxacin and norfloxacin, resistant strains ( $p<0.0001$ ). Finally, 50.8% (32/63) of fluoroquinolone-sensitive strains belonging to the phylogenetic group B2 were positive for *clb* genes ( $p<0.0001$ ).

**FEMS-0554**

**Bacterial pathogenicity**

## **MORAXELLA CATARRHALIS TARGETS HOST COLLAGENS FOR ADHERENCE**

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### **Background**

*Moraxella catarrhalis* is a human respiratory pathogen that causes acute otitis media in children and is a common cause of exacerbations in patients with chronic obstructive pulmonary disease. The first step for *M. catarrhalis* colonization is adherence to host tissues that is achieved by interaction with host extracellular matrix (ECM) proteins.

### **Objectives**

Characterization of the interaction between *M. catarrhalis* with host collagens present in respiratory system.

### **Methods**

Collagen I – VI were analysed for their interaction with *M. catarrhalis* clinical isolates by using ELISA and a radioactive direct binding assay. Collagen binding capacity of *M. catarrhalis* was also verified by coating collagens on the glass surface, followed by subsequent bacterial binding and detection by Gram staining. Molecular interaction between different human tissues/collagens and bacterial proteins were performed by EM.

### **Conclusions**

Ubiquitous surface protein (Usp) A2 and UspA2 hybrid (UspA2H) of *M. catarrhalis* were identified as major collagen interacting proteins. The mutants of UspA2 and UspA2H exhibited defective collagen interaction and did not recognize collagen coated on glass surface. All clinical isolates showed binding capacity to fibrillar collagens type I, II and III, and network forming collagen IV and VI. Only 20% clinical isolates showed collagen IV binding capacity. Our data suggests that UspA2/A2H based interaction to collagens could be critical for adhesion of *M. catarrhalis* to host tissues and plays an important role in pathogenesis.

**FEMS-1509**

**Bacterial pathogenicity**

## **PLASMID ENCODED VIRULENCE TRAITS IN CRONOBACTER SAKAZAKII**

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### **Background**

*Cronobacter* spp. are opportunistic pathogens that cause severe infantile meningitis, septicemia, or necrotizing enterocolitis. Contaminated powdered infant formula has been implicated as the source of this pathogen in most cases, but questions still remain regarding the likely habitat and virulence potential for this strain.

### **Objectives**

The motive of this study was to determine the occurrence of putative plasmid-harbouring virulence traits in *C. sakazakii*.

### **Methods**

The isolation was done as per ISO 22964:2006 on chromogenic media followed by further confirmation by phenotypic and genotypic characterization. Two putative virulence genes *Cronobacter* plasminogen activator (*cpa*) and ferric-iron transporter *eitABCD* gene (*eitA*) were detected by PCR. The *cpa* gene is reported to provide serum resistance to *C. sakazakii* whereas *eitA* gene is generally associated with iron uptake mechanism especially from breast milk and infant formula by *Cronobacter* spp. which may enhance its spread and invasion in a host.

### **Conclusions**

In our investigation, 15 *C. sakazakii* isolates were identified from 154 food and environmental samples based on phenotypic and genotypic characterization. PCR screening using *eitA*-specific primers revealed that the majority (67%) of the *C. sakazakii* isolates harbor the *eitA* gene while in contrast only 27% of the isolates were positive for the gene *cpa*. Taken together, these properties may contribute to the systemic survival of *C. sakazakii* and subsequent invasion of the central nervous system to cause disease. The current study clearly indicates that foods of plant origin are one of the most possible natural reservoirs of this pathogen and also provide important insight into this virulence plasmid from an emerging pathogen





**FEMS-1867**

**Bacterial pathogenicity**

**INHIBITION OF THE LYTIC ACTIVITY OF THE ALTERNATIVE AND CLASSICAL COMPLEMENT PATHWAYS BY A SURFACE LEPTOSPIRAL ADHESIN OF LEPTOSPIRA INTERROGANS**

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**Background**

Pathogenic *Leptospira* are the etiological agent of leptospirosis, a disease of human and veterinary concern. The ability of pathogenic leptospires to survive the bactericidal activity of human sera was demonstrated. Recently, it has been shown that leptospires acquire soluble regulators to evade the immune system.

**Objectives**

To assess the effect of the leptospiral adhesin Lsa23 on the activity of complement system.

**Methods**

The gene LIC11360 was cloned and the protein Lsa23 expressed in *Escherichia coli*. The effect of Lsa23 on classical pathway of complement was assessed by hemolytic assay using antibody-coated sheep erythrocytes, while the effect on alternative pathway was evaluated measuring the deposition of C5b9 on zymosan in presence of MgEGTA buffer. Interaction with factor H (FH), C4BP and plasminogen was evaluated by ELISA. Cofactor activity of complement regulators and proteolytic activity of plasmin were assessed by immunoblotting.

**Conclusions**

Lsa23 was expressed and purified successfully, as visualized by SDS-PAGE. It has been able to inhibit classical pathway-mediated hemolysis by 83%, and also blocked the alternative pathway reducing C5b-9 deposition by 90%. The interaction of Lsa23 with soluble C4BP, FH and plasminogen was dose-dependent. Competition assays suggest these components have distinct binding sites on Lsa23. C4BP and FH preserved their cofactor activity for factor I when bound to Lsa23, as demonstrated by cleavage of C4b and C3b, respectively. Plasminogen bound to Lsa23 could be converted into plasmin and degraded C3b and C4b. Thus Lsa23, a surface receptor for human plasma components, prevents the lytic activity of complement system and could contribute to leptospiral immune evasion process.

**FEMS-0932**

**Bacterial pathogenicity**

**THE VIRULENCE EFFECT ON A HIGHLY CONSERVED REGION OF GENES RESPONSIBLE FOR CAPSULAR POLYSACCHARIDE SYNTHESIS IN KLEBSIELLA PNEUMONIAE**

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**Background**

Capsular polysaccharides (CPS) are one of the major virulent determinant in *Klebsiella pneumoniae* (KP). Among different serotype of KP, CPS gene cluster could be distinguished into two regions, a conserved and a hyper-variable region.

**Objectives**

Virulence's study by genes' knockout, phagocytosis, serum resistance and mice lethality on highly conserved region of genes in CPS cluster was performed.

**Methods**

Serotype K20 KP was selected. Six genes including *galF*, *acidPPc*, *wzi*, *wza*, *wzb* or *wzc* in CPS conserved region were knocked out and assessed their effect on virulence. In comparing to parental K20 isolate, mutants showed a varied decline in mice lethality (LD<sub>50</sub>) from 10 fold to > 10<sup>5</sup> fold and could be categorized into low (L), moderator (M), and High (H) effect on virulence. For polycistronic mRNAs driven by P1 promoter consisted *galF* and *acidPPc*, low effect on serum resistance and anti-phagocytosis was observed. Only 10 fold of decreasing in mice lethality was achieved in the *DgalF* and *DacidPPc* mutants. For the rest of *wzi*, *wza*, *Dwzb* or *Dwzc* that were driven by P2 promoter, disruption of capsule surface assembly, *Dwzi*, had moderate effect with reduced mice lethality for 100 fold. The deletion of genes, *Dwza*, *Dwzb* or *Dwzc* which were involved in CPS polymerization, caused a significant decrease on virulence (LD<sub>50</sub> >10<sup>7</sup>) and became phagocytic susceptible indicating the importance of these genes in synthesis of capsule.

**Conclusions**

Although conserved genes in CPS cluster were all involved in KP-CPS synthesis, they contributed differently in virulence.

**FEMS-0834**

**Bacterial pathogenicity**

**UNRAVELING THE FUNCTION OF A LEE-ENCODED ORF: ESCK (ORF4) IS A STRUCTURAL COMPONENT OF THE TYPE III SECRETION SYSTEM OF ENTEROPATHOGENIC ESCHERICHIA COLI**

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**Background**

The type III secretion system (T3SS) is a molecular device employed by many pathogenic bacteria to translocate a set of proteins, called effectors, directly into the cytoplasm of eukaryotic cells in order to manipulate normal cellular processes to the benefit of the bacterium.

Enteropathogenic *Escherichia coli* (EPEC), an important causative agent of infant diarrhea, relies on a T3SS to deliver effectors into intestinal enterocytes allowing it to colonize the host gastrointestinal tract. Structural components assembling this biological machinery are encoded on a chromosomal pathogenicity island named the *locus of enterocyte effacement* (LEE).

Although the vast majority of LEE-encoded proteins have an assigned function, there are still few of them uncharacterized. One such example is the protein encoded by the *escK* gene (formerly *orf4*).

**Objectives**

To elucidate the role of the LEE-encoded protein EscK in T3SS biogenesis and function.

**Methods**

An EPEC  $\Delta$ *escK* null mutant was generated to assess its type III secretion phenotype. An extensive search of EscK protein-protein interactions with components of EPEC T3SS was performed using the yeast two-hybrid system. Novel protein interactions were corroborated by pull-down experiments. Bacterial fractionation experiments and site directed mutagenesis were carried out in order to dissect the molecular function of this protein.

**Conclusions**

EscK is a critical component of the EPEC T3SS, it is localized to the inner membrane and its localization does not depend on other T3SS components. EscK interacts directly with the C-ring/sorting platform component EscQ. Essential residues for EscK function were identified.



**FEMS-1350**

**Bacterial pathogenicity**

## **IRON UPTAKE SYSTEMS AMONG ESCHERICHIA COLI ISOLATED FROM SKIN AND SOFT-TISSUE INFECTIONS**

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### **Background**

*Escherichia coli* is a commensal bacterium that can be found in the intestinal microbiota. However, pathogenic *E. coli* strains that are associated with numerous infections, possess virulence factor (VF) genes. *E. coli* is known to be an important cause of skin and soft-tissue infections (SSTI) as it is the 3<sup>rd</sup> most prevalent isolated species from SSTI nevertheless, *E. coli* from such infections are rarely investigated.

### **Objectives**

The main goal of our research was to shed more light on certain iron uptake systems among SSTI *E. coli* strains. We determined their prevalence and analysed possible correlations between their genes and phylogenetic groups and some other VFs as well as bacteriocin genes.

### **Methods**

A collection of 102 previously described SSTI *E. coli* strains was used. The methods employed were cultivation in LB medium, preparation of lysates, and polymerase chain reaction (PCR) with primers specific for the investigated target genes (*fyuA*, *iroCD*, *iucD*, *iha*, *ireA*, *picU* and *hbp*). The PCR products were detected and visualised with agarose gel electrophoresis. All results were statistically processed using Fischer's exact test and Bonferroni correction.

### **Conclusions**

The prevalence of iron up-take systems among the studied collection was 76%, 60%, 47%, 30%, 19%, 10% and 5% for *fyuA*, *iroCD*, *iucD*, *iha*, *ireA*, *picU* and *hbp*, respectively. The following correlations were observed: *fyuA* with B2 phylogenetic group and *cnf1*, *hlyA*, *kpsMTII*, *ompT* and *usp* genes; *hbp* with *ompT*<sub>APEC</sub> and microcin V; *ireA* with *papGII*; *iha* with *papGII* and *iucD*; and *iroCD* with microcinH and *sfa*.



**FEMS-1588**

**Bacterial pathogenicity**

## **MECHANISMS OF NEWBORN BRUCELLA ABORTUS INTERNALIZATION IN CULTURED HOST CELLS.**

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### **Background**

Bacteria from the *Brucella* genus are gram negative intracellular pathogens responsible for Brucellosis, one of the most widespread anthro-po-zoonosis worldwide. *Brucella* spp. are intracellular pathogens and despite their tremendous impact on world health and economics no human vaccine is currently available and little is known about molecular mechanisms underlying the infection process.

*Brucella* infection of cultured host cells (*i.e.* HeLa cells and RAW 264.7 macrophages) is biphasic. It is first characterized by a « lag phase » consisting in a very low but constant number of colony-forming units during the first hours of infection, and followed by a second « duplication phase » during which bacteria proliferate massively. This defines adhesion/invasion of host cells as a critical step for successful infection.

Recent data showed that infection is mainly carried out by a bacterial subpopulation composed of newly generated bacteria called « newborns », characterized by a single genome copy (Deghelt, Mullier *et al.* 2014)

### **Objectives**

Identification of bacterial components mediating preferential adhesion to and/or invasion of cultured host cells by newborn *Brucella*.

### **Methods**

'With *a priori*' gene deletion, 'without *a priori*' transpositional mutagenesis screen (Tn-seq), lectin staining.

### **Conclusions**

So far, three adhesins (BmaC, BtaE, BtaF) have been shown not to act in the newborn selection when infecting HeLa cells and RAW 264.7 macrophages. Tn-seq is still ongoing. We have detected specific binding of the wheat germ agglutinin to the new pole of *B. abortus* when grown until stationary phase in rich medium.





**FEMS-2751**

**Bacterial pathogenicity**

**INTERACTION WITH VITRONECTIN, LAMININ AND FIBRONECTIN: A NOVEL  
ROLE OF *Haemophilus influenzae* LIPOPROTEIN P4 IN SERUM RESISTANCE  
AND ADHERENCE TO PULMONARY EPITHELIAL CELLS**

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Background Nontypeable *Haemophilus influenzae* (NTHi) is a human-specific Gram-negative species that primarily colonizes the nasopharyngeal and respiratory tract. Interaction with extracellular matrix (ECM) proteins is one of the colonization strategies described for NTHi, and involves several bacterial adhesins.

Objective We describe herein the identification and functionality characterization of a NTHi multifunctional ECM protein receptor.

Methods Outer membrane proteomic analysis revealed that *Haemophilus* outer membrane protein 4 (P4; an acid phosphatase involved in the uptake of nicotinamide adenine dinucleotide) interacted with multiple ECM proteins including vitronectin, laminin and fibronectin. This was further validated when an NTHi 3655 mutant devoid of P4 (NTHi3655 $\Delta$ hel) significantly lost binding to the ECM proteins. Binding was restored by a hel gene trans-complemented mutant. Analyses of protein-protein interactions by ELISA revealed that purified recombinant P4 has a high binding affinity for laminin (dissociation constant,  $K_D=9.26$  nM) and fibronectin ( $K_D=10.19$  nM), but slightly less to vitronectin ( $K_D=16.51$  nM). Interestingly, binding to the ECM proteins was optimal with dimeric P4, a structure that is dependent on the  $\alpha$ -domain. Vitronectin acquisition conferred serum resistance to both P4-expressing NTHi 3655 and *Escherichia coli* transformants, but not to the P4-deficient strains. Importantly, NTHi3655 $\Delta$ hel also displayed decreased adherence to both type II alveolar epithelial and pharynx epithelial cells, and blocking with antibodies suggested the P4-mediated cell adherence was mainly attributed to fibronectin.

Conclusions In conclusion, our data provides new insight into the potential role of P4 as a multiple ECM protein receptor important for colonization and establishment of NTHi infection.

**FEMS-3111**

**Bacterial pathogenicity**

**PASTEURELLA PNEUMOTROPICA SEPTICEMIA IN AN ELDERLY WOMAN**

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**Background**

*Pasteurella penumotropica* (PP) is a small gram (-) cccobacillus which is a normal inhabitant of the oropharynx of small animals. Human infections due to PP rarely occur. We saw an elderly woman with septicemia due to PP, and describe herein .

**Objectives**

Human infections due to PP are rarely seen, and it is our intention to share the case experience with others.

**Methods**

A 76 yo woman with comorbidities including ESRD on hemodialysis, DM, CVA, and hypertension was admitted for evaluation of fever, chills, sore throat and fatigue. She also complained of increased cough with yellowish sputum production. She denied myalgia, arthralgia, and dyspnea. She owns a dog. On exam. temp., 38.9C, RR, 23/min, and PR, 112/min. Other positive findings were: erythematous pharynx without exudate, lung auscultation showed diffuse bilateral rhonchi. Lab. findings: leukocytes, WBC at 31.7K/cmm; BUN 43 mg/dl; and Cr 8.69 mg/dl. Sputum culture grew normal oral flora. Blood cultures (B/C) obtained on admission revealed positive (4/4) for small gram-negative coccobacilli which was confirmed to be PP. Antibiotic susceptibility testing: sensitive to penicillin containing preparations (i.e., ampicillin, amoxicillin/clavulanate), doxycycline, TPM/SMX, and moxifloxacin, while resistant to ceftriaxone and azithromycin. She was initially begun on amp./sulb. and she became afebrile and abacteremic on day 3. She remained abacteremic afterward and was discharged on amox/clav 875 mg po BID to complete a 2-wk course.

**Conclusions**

A patient with no known history of definite animal bite or scratch developed PP septicemia and she responded to penicillin preparation nicely without further complication.

**FEMS-2570**

**Bacterial pathogenicity**

**STUDY OF THE AVIAN SALMONELLA CONTRIBUTION TO HUMAN  
SALMONELLOSIS IN CHAD: EXAMPLE OF THE CAPITAL, N'DJAMENA**

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**Background**

*Salmonella* infection is a major health concern and continues to have a serious economic impact worldwide. It is estimated that *Salmonella* serotypes cause 93.8 million human infections and 155,000 deaths annually through the world.

**Objectives**

The main objective of this study was to update the prevalence and antimicrobial resistance characteristics of *Salmonella* isolated from poultry and from humans experiencing gastroenteritis in N'Djamena and also to evaluate the phylogenetic relationships and to find epidemiological links between human and avian strains isolated in the same period of time and in the same region.

**Methods**

All samples (droppings, sterile Cloths, food and water) collected were analysed according to French Norm for *Salmonella* Spp. NF ISO 6579/2002.

A total of *Salmonella* isolates found in humans and poultry in this study were characterised by ERIC-PCR and IS200-PCR. The PCR analysis was followed by macrorestriction analysis (PFGE).

**Conclusions**

Diagnostic methods carried out during this study led to the isolation of one hundred and twenty seven *Salmonella* strains, belonging to forty two different serotypes. *Salmonella* Colindale was the most prevalent serovar (13.6 %), followed by *Salmonella* Minnesota (10.8 %), *Salmonella* Stanleyville (5.8 %).

PCR results demonstrated that, in the context of this epidemiological study, ERIC- and IS200-PCR methods can be used effectively to limit the number of isolates that have to be serotyped. The comparison of ERIC-PCR, IS200-PCR, PFGE and antimicrobial susceptibility profiles among isolates from human and avian origins has highlighted, the indirect evidence of human contamination sources by *Salmonella* serovars from poultry farms.



**FEMS-1943**

**Bacterial pathogenicity**

**QUANTIFICATION OF LIPOTEICHOIC ACID (LTA) IN ROOT CANALS WITH NECROTIC PULP SUBJECTED TO ENDODONTIC TREATMENT**

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**Background**

Gram-positive bacteria release lipoteichoic acid (LTA) as an important virulence factor, which is structurally and immunologically similar to lipopolysaccharide (LPS). The knowledge about LTA actions *in vivo* remains unclear, as well as their participation in the etiology of pulp and periapical diseases, with no reported studies on the quantification of LTA in root canals.

**Objectives**

a) quantifying the levels of LTA in primary infections of root canals with necrotic pulp and apical periodontitis; b) verifying the effects of biomechanical preparation using sodium hypochlorite 2.5% in LTA reduction in root canals.

**Methods**

Ten single-rooted teeth of patients needing endodontic treatment were prepared with Reciproc rotation system and irrigated with 2.5% NaOCl. Root canals samples were obtained immediately after coronary opening (S1) and after biomechanical preparation (S2). LTA quantification was performed by enzyme immunoassay (ELISA), with specific anti-LTA antibodies. The optical density values were converted to µg/mL and analyzed by Student's t test (significance level of 5%).

**Conclusions**

Results: All root canals showed LTA in the collection S1 with an average of  $119.4 \pm 73.2$  µg/mL. After instrumentation, it was observed a reduction in the levels of LTA, with an average in the collection S2:  $77.6 \pm 22.4$  µg/mL.

Conclusion: The root canals with necrotic pulp showed high levels of LTA and only instrumentation with sodium hypochlorite 2.5% was not enough for its entirely

elimination of the root canals. New studies with other endodontic treatment protocols should be performed for new findings.

**FEMS-2661**

**Bacterial pathogenicity**

**CHARACTERIZATION OF TWO PROTEINS OF LEPTOSPIRA INTERROGANS WITH POTENTIAL ROLE IN HOST-PATHOGEN INTERACTIONS**

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**Background**

Leptospirosis is a worldwide zoonosis regarded as a major public health problem. Measures to control the disease are difficult to implement. The development of new strategies to prevent and control the spread of disease is urgently needed. Accordingly, vaccines emerge as strong candidates to solve the problem. For this reason, currently research has focused to identify conserved antigens that are involved in host-pathogen interactions.

**Objectives**

Evaluate the functional properties of the coding sequence LIC13479 and LIC10050 of *L.interrogans* serovar Copenhageni, identified by bioinformatics as putative outer membrane proteins.

**Methods**

The gene sequences were cloned into the expression vector pAE. Plasmids containing cloned DNA were introduced in *E. coli* strains for protein expression. After purification of the recombinant proteins, mice were immunized for polyclonal antibodies production. Reactivity of the recombinant proteins was evaluated in serum samples of leptospirosis patients and of febrile unrelated diseases. The ability of recombinant proteins to interact with the host by adhering to extracellular matrix proteins or serum components was examined.

**Conclusions**

The coding sequences LIC13479 and LIC10050 were cloned and expressed successfully. Evaluation of the purified recombinant proteins showed that they are capable to stimulate antibody immune response in mice and, in addition, they are recognized by infected human serum samples. Both recombinant proteins exhibited adhesin properties and, in addition, interacted with plasminogen and can generate plasmin in the presence of activator. Our data indicate that these proteins could promote the attachment and contribute to the invasion processes within the hosts

**FEMS-1155**

**Bacterial pathogenicity**

**MOLECULAR CHARACTERIZATION OF SHIGA TOXIN-PRODUCING  
ESCHERICHIA COLI (STEC) ISOLATED IN 2012 IN THE COUNTRY OF GEORGIA**

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**Background**

STEC-associated HUS was first recognized in Georgia in 2009 following the diagnosis of a cluster of HUS cases in Georgia.

**Objectives**

In this study we genetically characterized two sporadic cases (Case A and B) of HUS syndrome occurred in 2012. Among them was one family case reported (Case B), where four individuals revealed positive on STEC, but only one developed HUS.

**Methods**

Clinical samples were enriched in broth and simultaneously plated on selective agar media. DNA was extracted and tested by two different conventional Multiplex PCR assays for final confirmation of STEC (*stx1*, *stx2*, *eae*, *Ehly*) and detection O104 strain markers (*stx2*, *terD*, *rfbO104*, *fliC H4*). Furthermore, for molecular genotyping Pulsed Field Gel Electrophoresis (PFGE) was applied. Five STEC strains were isolated from both described cases. All revealed positive results by molecular tests on STEC but showed different genetic profiles based on combination of existing



toxigenic markers. Interestingly, it was observed that case A tested positive on all four O104 specific genes. Besides, PFGE typing showed two different genetic profiles as well.

## **Conclusions**

Here described in both HUS cases, a diversity of STEC isolates was detected. However, one of the causative agents was considered to be STEC O104. It is remarkable that two O104 strains were isolated during STEC outbreak in Georgia in 2009 which are genetically related with the strains isolated during STEC outbreak in Germany in 2011. This finding suggests that O104 strain has spread and been circulating for several years in the country.

**FEMS-1861**

**Bacterial pathogenicity**

**ROLE OF IRON IN THE CROSS-TALK BETWEEN THE BIOSYNTHETIC PATHWAYS LEADING TO THE PRODUCTION OF SIDEROPHORES AND COLIBACTIN.**

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**Background**

The biosynthetic pathways of several siderophores and the genotoxin colibactin require the enzymatic activity of a phosphopantetheinyl transferase (PPTase). We have shown that ClbA, the PPTase associated with the production of colibactin, can also contribute to the production of siderophores, whose production is dependent on the bioavailability of iron in the medium, through the regulation of the expression of EntD, the PPTase previously thought to be the unique PPTase involved in the production of siderophores in *E. coli*.

**Objectives**

We hypothesized that iron could also modulate the production of colibactin through the transcriptional regulation of *clbA*.

**Methods**

Mutant derivatives of *E. coli* were engineered. We investigated transcription of genes involved in the production of colibactin in iron depleted or repleted conditions using qRT-PCR and luciferase reporter gene assay. Quantification of total siderophores was assessed.

**Conclusions**

This study revealed an increased transcription of *clbA* in iron limiting conditions, and a decrease of *clbA* expression in iron supplemented media. Quantification of total siderophores revealed that the increased expression of *clbA* in iron-depleted conditions was associated to an increased synthesis of siderophores. Mutation of *entD* leads to an exacerbation of the induction of *clbA* expression, whereas overexpression of *entD* or *clbA* leads to a repression of *clbA* transcription. Analysis of the region upstream *clbA* shows the presence of two putative Fur-boxes, which could explain its regulation by iron. In conclusion *clbA* is tightly regulated by iron

bioavailability leading to an increased production of siderophores that could explain the high pathogenicity of the strains synthesizing colibactin.

**FEMS-0572**

**Bacterial pathogenicity**

## **VIRULENCE FACTORS OF STREPTOCOCCUS PYOGENES ISOLATED FROM PATIENTS WITH TONSILLOPHARYNGITIS IN KONYA/TURKEY**

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### **Background**

*Streptococcus pyogenes* is an important bacterial pathogen that colonizes the throat and skin of a host and produces a wide variety of virulence factors such as toxins, proteases or DNases.

### **Objectives**

The aim of this study was to investigate the antibiotic resistance and virulence factors (*speA*, *B*, *C*, *G*, *H*, *I*, *J*, *K*, *L*, *M*, *smeZ*, *ssa*, *spd3*, *sdc*, *sdaB*, *sdaD*, *spyCEP*, *scpA*, *mac* and *sic*) of *S. pyogenes* strains isolated from throat cultures of patients with symptomatic tonsillopharyngitis.

### **Methods**

One hundred and fifty *S. pyogenes* isolates were identified by conventional methods and VITEK 2 automated system. Antibiotic susceptibility tests were performed by Kirby-Bauer disk diffusion method as recommended by Clinical and Laboratory Standards Institute. The virulence factors were determined by multiplex PCR.

### **Conclusions**

All of the *S. pyogenes* isolates were susceptible to penicillin G, cefotaxime, ceftriaxone, chloramphenicol, clindamycin, erythromycin, levofloxacin, vancomycin and linezolid. Among streptococcal pyrogenic exotoxin genes the most frequent gene was *speG* (88.0%) following *speC* (59.3%), *smeZ* (46.7%), *ssa* (42.7%), *speA* (33.3%), *speJ* (24.0%), *speK* (18.7%), *speH* (14.0%), *speI* (13.3%), *speL* and *speM* (9.3%). From DNases, proteases and inhibitors *sdaB*, *speB*, *spyCEP*, *scpA* and *mac* were positive in all strains, and *spd3*, *sdc*, *sdaD* and *sic* were carried 64.7%, 36.0%, 24.7%, 2.0% of the isolates respectively. In conclusion, *S. pyogenes* isolates collected from throat cultures of patients with symptomatic tonsillopharyngitis in Konya/Turkey possess high virulence factors, and susceptible to antibiotics.

**FEMS-1518**

**Bacterial pathogenicity**

**COMBINATION BETWEEN A FEW T3SS INJECTISOME AND A LOT EFFECTOR FOR KILLING HOST CELLS ON VIBRIO PARAHAEMOLYTICUS**

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**Background**

Type 3 secretion system (T3SS), is major virulent factor in over twenty species of Gram-negative bacteria which export bacterial protein (effectors) to host cell to manipulate its cellular function for own's hope. Moreover, T3SS is a prospective useful tool with both experimental and therapeutic applications, including vaccine development.

**Objectives**

Upon analysis of the contribution of the T3SS injectisome and effector involved in this paradigm, it is still unknown that the number and timing of expression of the T3SS injectisome and effectors, and which factor have dominant role during infection are unclear. In the present study, we used fluorescence protein combined with single cell analysis to solve dynamics of the T3SS and effector during infection in *Vibrio parahaemolyticus*.

**Methods**

During infection, Vp1671 (T3SS component) but not the Vp1680 (effector) proteins showed to make spot like localization along the membrane of the cell. Although both percentages of spot positive cells and number of T3SS spots were increased depend on a time of infection, assemblies of the Vp1671 to the T3SS occur prior to increase fluorescence levels of bacterium in early time of infection. Cytotoxicity against the host cells were increased depend on the expression levels of Vp1680 and number of T3SS spot, if it is only one spot.

**Conclusions**

*V. parahaemolyticus* use a combination between a few T3SS injectisome regulated by localization and a lot effector regulated by expression for killing host cells.

**FEMS-1475**

**Bacterial pathogenicity**

**ASYMPTOMATIC BACTERIURIA STREPTOCOCCUS AGALACTIAE:  
MOLECULAR CHARACTERIZATION AND IMPLICATIONS FOR PATHOGENESIS**

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**Background**

*Streptococcus agalactiae* causes urinary tract infection (UTI) including asymptomatic bacteriuria (ABU); however, growth of *S. agalactiae* in urine has not been reported and the role of this growth phenotype in human infection is unknown.

**Objectives**

We characterised the ability of different *S. agalactiae* UTI isolates to grow in urine, and defined the molecular mechanism(s) of growth and implications for human ABU.

**Methods**

We used growth assays, competition assays, metabolic phenotype arrays, whole-genome sequencing, and mutation analysis in *S. agalactiae*. We report robust growth of ABU *S. agalactiae* (ABSA) in human urine that was not seen among uropathogenic *S. agalactiae* (UPSA) isolated from patients with acute UTI. In competition assays using a prototype ABSA strain, designated ABSA 1014, and any one of several UPSA strains, we observed markedly superior fitness of ABSA 1014 for urine growth. Phenotype profiling of ABSA 1014 and UPSA 807, isolated from a patient with cystitis, using metabolic arrays revealed specific L-Malic acid catabolism in ABSA 1014 that was absent in UPSA 807. Whole-genome sequencing revealed divergence in malic enzyme-encoding genes between the strains predicted to impact the activity of malate metabolism. Urine growth assays comparing wild-type ABSA and mutants that were functionally inactivated for malate metabolism by disruption of *maeE* or *maeK* demonstrated attenuated growth of the mutants in urine and synthetic human urine containing malic acid.

**Conclusions**

We conclude that some ABU *S. agalactiae* can grow in urine, and this relates in part to malic acid metabolism, which may affect *S. agalactiae* UTI.

**FEMS-1053**

**Bacterial pathogenicity**

## **THE ROLE OF THE MYCOSIN PROTEASE IN TYPE VII SECRETION OF PATHOGENIC MYCOBACTERIA.**

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### **Background**

A promising new group of novel drug targets for *Mycobacterium tuberculosis* are the type VII secretion (T7S) systems. *M. tuberculosis* has five of these systems, ESX-1 to ESX-5, of which at least three are crucial for the virulence and/or viability of this pathogen. One of the conserved components of T7S systems is mycosin (MycP), a membrane-associated protease that is an essential part of the T7S system. Due to the proteolytic activity of the mycosins they are promising targets for novel drugs against TB.

### **Objectives**

We set out to functionally dissect the role of the mycosin proteases in T7S, by analyzing the phenotype of MycP1 and MycP5 mutants.

### **Methods**

Deletion strains of the mycosins of the ESX-1 and ESX-5 secretion systems were created in *Mycobacterium marinum*. Subsequently, proteolytic inactive versions of MycP1 or MycP5 were introduced in the deletion strains. Functional complementation by these variants was verified by the effect on the secretion by the ESX-1 and ESX-5 systems.

### **Conclusions**

While deletion strains of MycP1 or MycP5 were defective in respectively ESX-1 or ESX-5 dependent secretion, the protease inactive variants were able to mediate secretion by ESX-1 or ESX-5. Thus the proteolytic activity of MycP is not essential for its respective ESX-system dependent secretion. These results indicate a dual function for mycosins, with a proteolytic role in substrate processing and a second, so-far unknown, role in the regulation of the secretion process. We are currently

unravelling this second function in T7S and pinpointing which domain of MycP is involved in this process.



**FEMS-1868**

**Bacterial pathogenicity**

**EFFECT OF DIFFERENT FACTORS ON THE (TWITCHING) MOTILITY OF  
PHOTOBACTERIUM DAMSELAE SUBSP. PISCICIDA.**

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**Background**

*Photobacterium damsela* subsp. *piscicida*, which is the etiological agent of photobacteriosis and affects different species of sea fish, is a non-motile Gram-negative bacterium. Notwithstanding, it is able to move on solid surfaces. Because of the presence of pili-like structures on the bacterial surface, discovered the last year by Remuzgo-Martínez *et al.*, and being associated with twitching motility,

**Objectives**

the objective of this study was to modify some conditions (temperature, pH, concentration of nutrients, salinity, nature of the medium and others) to determine which ones promote motility.

**Methods**

A total of five bacterial strains were tested: C2, 94/99, DI21, PP3 and ATCC 17911. The results of motility assays were obtained after two and three days of inoculation in “twitching motility” medium and statistically analyzed with IBM SPSS Statistics 22.0 program.

**Conclusions**

Lower temperatures, alkaline and acidic environments and lacking salinity had a negative effect on cell motility, while nutrient limitation did not affect the bacterial response. The best twitching motility was observed when *Phdp* was inoculated in medium with 0.2% agar and pH 7.0. Also, interesting results were achieved after scraping petri dishes (surface where the bacteria moved) or the addition of cellular debris of SAF-1 cell line to the medium.

**FEMS-2211**

**Bacterial pathogenicity**

**IDENTIFICATION AND DISTRIBUTION OF A NOVEL 22-KDA OUTER MEMBRANE PROTEIN RELATED TO SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) STRAINS NEGATIVE FOR LOCUS ENTEROCYTE EFFACEMENT.**

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**Background**

STEC strains are etiologic agents of acute diarrhea, bloody diarrhea and hemolytic uremic syndrome (HUS). Associated with STEC outbreaks and HUS, we may observe two main groups of strains, those that in their genomes is encoded the Locus Enterocyte Effacement (LEE-positive), including the serogroups, O157:H7, O26, O103 and O111, and bacteria without this Locus (LEE-negative) which include the serogroups, O113 and O91: H2.

**Objectives**

Describe a novel antigen present in the outer membrane (OM) of a LEE-negative STEC strain.

**Methods**

By western blot and immunoproteomic analysis (2D electrophoresis - MALDI-TOF/TOF) using sera from patients who developed HUS, we were able to identify an immunoreactive protein of 22 kDa with an isoelectric point of 5.0. By mean of bioinformatics tools, we found the encoded gene in the genome of STEC strain O91: H21 B2F1 LEE-negative (Accession: AFDQ01000026.1). Using specific primers, the gene was amplified and its presence was studied in a collection of 170 STEC strains and 11 commensal *E.coli*. Interestingly, the gene was detected only in LEE-negative STEC strains (63%; 32/51). Sequencing of PCR products showed that this gene is highly conserved. Preliminary results suggest that this protein could be involved in bacterial adhesion and hemagglutination.

**Conclusions**

Our results suggest that the identified antigen might be associated only with a subset of LEE-negative STEC strains. Additional studies are needed to determine the role of this protein in STEC pathogenicity.

**Funded:**FONDECYT1110260

**FEMS-1754**

**Bacterial pathogenicity**

**LEPTOSPIRA INTERROGANS STIMULATES PLASMINOGEN ACTIVATORS IN VIVO IN EXPERIMENTALLY INFECTED ANIMALS AND NATURALLY ACQUIRED HUMAN LEPTOSPIROSIS.**

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**Background**

Leptospirosis is a globally important zoonosis, caused by pathogenic bacteria of genus *Leptospira*. The molecular mechanisms for leptospiral pathogenesis and virulence remain poorly understood. A pathogen invasion, dissemination and host tissue damaging depend on proteolytic enzymes both of the invading organism and of the host. Many pathogens express their own proteases or exploit hosts proteases to activate other protease-dependent cascade systems. We have described that leptospires capture plasminogen (PLG) on the outer surface, which is converted to plasmin (PLA) by exogenous activators. We also showed that leptospires induce the expression of PLG activators by human endothelial cells *in vitro*.

**Objectives**

Here, we further characterized the interactions of *L. interrogans* with human PLG/PLA system *in vivo*.

**Methods**

We studied the stimulation of PLG activators in different time points during leptospirosis in hamsters infected with lethal doses, and in human sera samples from the initial and convalescent phase of the disease.

**Conclusions**

Our data indicate that leptospirosis human patients have increased levels of circulating urokinase-type and tissue-type PLG activators when compared to normal sera, being especially augmented at the early phase of the disease. In the sera of experimentally infected hamsters, increasing levels of PLG activators were observed during the progression of the infection, until euthanasia. Additionally, hamsters' organs extracts similarly showed increased PLG activators activity.

The results presented here further characterize the host response to the *Leptospira* infection, in the light of PLG/PLA system stimulation. Our data strengthen the importance of the fibrinolytic system to the leptospirosis infectious process.

**Support: FAPESP, CNPq, Fundação Butantan.**



**FEMS-1750**

**Bacterial pathogenicity**

**BINDING OF LEPTOSPIRA INTERROGANS TO HUMAN THROMBIN,  
BIOLOGICAL IMPLICATIONS AND IDENTIFICATION OF POSSIBLE BACTERIAL  
LIGANDS**

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**Background**

Leptospirosis is a zoonosis caused by pathogenic spirochete of genus *Leptospira*. The symptoms range from a mild fever to a more complicated, severe form of the disease, called Weil's syndrome. Since the 1980s, the incidence of severe pulmonary hemorrhage caused by *Leptospira* spp. infection has increased. The reasons behind the emergence of this syndrome are not known. Many pathogens can interfere in the coagulation cascade by binding to fibrinogen or by interaction with the enzyme thrombin.

**Objectives**

To characterize the interaction of leptospiral strains to thrombin, analyze the possible biological relevance and select possible proteins that could mediate these interactions.

**Methods**

The interaction of pathogenic (virulent and attenuated) and saprophytic strains with thrombin was performed by ELISA and western blotting. Characterization of the binding sites in thrombin molecule was accessed by co-incubation with different competitors. Reduction in thrombin activity was evaluated by fibrin clot formation assay. Identification of ligands was performed by ELISA by employing a set of recombinant leptospiral proteins.

**Conclusions**

We demonstrated that the pathogenic strains of *Leptospira* display a prominent binding to thrombin, particularly the virulent one. Competition assays indicate that virulent strain binds thrombin via exosite I. Virulent strain could reduce thrombin activity. Although we have identified some proteins that displayed binding to thrombin, none was capable to reduce the enzymatic activity. The interaction of virulent strains of *Leptospira* with the key enzyme of the coagulation cascade, thrombin, might constitute a novel mechanism of virulence, that could help understand the hemorrhagic features of leptospirosis.

**FEMS-1917**

**Bacterial pathogenicity**

## **RELATION BETWEEN THE SEQUENCE TYPE AND THE PROSPECTIVE VIRULENCE OF CRONOBACTER STRAINS**

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### **Background**

Bacteria from the genus *Cronobacter* are pathogens endangering all age groups, with severe clinical complications especially in neonates and infants. A multilocus sequence typing (MLST) scheme based on 7 housekeeping genes was constructed for *Cronobacter* spp. identification. The method also enables the recognition of sequence types (STs) and clonal complexes (CCs). Although 7 housekeeping genes (*atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB*, and *ppsA*) used for MLST analysis are not virulence related, connection between infections and particular sequence types were described in the literature.

### **Objectives**

The aim of this work was to evaluate the relation between sources of tested strains and their characteristics particularly their sequence type.

### **Methods**

MLST aimed to 7 genes (*atpD*, *fusA*, *glnS*, *gltB*, *gyrB*, *infB*, and *ppsA*) was performed on bacterial collection (over 80 strains). The amplification of genes *gltB* and *gyrB* showed to be difficult, and therefore new sets of primers were designed and tested.

### **Conclusions**

The target genes were sequenced in the majority of tested strains; in the case of incomplete information putative sequence type was determined. The obtained results showed widespread distribution of STs within tested strains. The significant predominance of particular STs was not determined in tested strains. Perhaps, the amount of tested strains was not representative enough; the ST relation to virulence will be further studied together with other possible factors.

This work was supported by the Czech Grant Agency (13-23509S) and Specific University Research (MSMT No. /2015).

**FEMS-1785**

**Bacterial pathogenicity**

## **GENOMICS AND PROTEOMICS IN A SEARCH FOR FACTORS IMPLICATED IN VIRULENCE OF STAPHYLOCOCCUS AUREUS**

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### **Background**

*Staphylococcus aureus* is an opportunistic pathogen of humans and animals. Our studies have revealed a link between the genotype of poultry-isolated *S. aureus* strains and their virulence in chicken embryo but not nematode model, what may suggest the existence of factors responsible for host preference and virulence.

### **Objectives**

Here we compare genomes and extracellular proteomes of four virulent and four non-virulent *S. aureus* strains in chicken embryo model. The open reading frames translation products for all strains were taken together and clustered according to the similarity level of 80%. Over three thousands clusters were obtained with only around 40% of clusters containing proteins with identical sequence across all strains. However, within the virulent and non-virulent group around 80% and 54% of clusters ORFs encoded identical proteins, respectively. The exoproteomes were highly heterogeneous irrespective virulence, however we were able to point alpha-hemolysin and bifunctional autolysin as indicators of virulence whereas glutamylendopeptidase as potential virulence attenuator.

### **Methods**

The genomes were obtained using MiSeq Illumina sequencer and assembled with MIRA and CLC Main Workbench software. Extracellular proteins were precipitated, labeled with fluorescent dyes and subjected to two-dimensional difference gel electrophoresis in pairs virulent *versus* non-virulent. Differentiating protein spots were subjected to mass spectrometry analysis.

### **Conclusions**

Deep sequencing confirmed genetic dissimilarity of virulent and non-virulent strains which finds its reflection in extracellular proteome. However, proteomics also points transcriptional and translational events as modulators of *S. aureus* virulence.

The study was supported by the grant UMO-2012/07/D/NZ2/04282 (to B.W.) from National Science Centre, Poland.





**FEMS-0621**

**Bacterial pathogenicity**

## **ADHESINS FROM YERSINIA RUCKERI**

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### **Background**

*Yersinia ruckeri* is a Gram-negative bacterium pathogen responsible for enteric redmouth disease (ERM). *Yersinia ruckeri* infections cause significant economic losses in salmonid aquaculture. ERM can be effectively controlled by antibiotic treatment and by application of whole cell based vaccines. Nevertheless, ERM outbreaks are still observed. More recently, vaccine resistant isolates caused disease throughout Europe. Research into the pathogenicity of *Yersinia ruckeri* is limited. Much more is known about the host-pathogen interaction of the three *Yersinia* species pathogenic to humans.

The group of proteins, that mediate the host-pathogen interaction comprise adhesins anchored in the bacterial outer membrane. They are essential for infection as they possess the ability to bind a variety of host molecules such as collagen, fibronectin, laminin,  $\beta 1$  integrins.

### **Objectives**

The aim of my research is to establish a fish infection model – zebrafish to understand the molecular mechanisms of adhesion and to identify adhesins of interest and their cellular receptors in order to assess their function in pathogenesis.

### **Methods**

To examine whether adhesins of interest are involved in *Yersinia ruckeri* virulence, fish cell line and in vivo system (zebrafish embryos) will be used and optimise. Fluorescence microscopy will allow to visualize infections to assess the role of virulence genes. Infection of colour- fluorescence bacteria will be essential to follow the infection.

### **Conclusions**

Understanding the molecular mechanisms of adhesion in fish, might prevent progression of infections. Moreover, the knowledge gained could help in developing new antimicrobials that could directly act as vaccines against bacterial fish diseases.

**FEMS-2497**

**Bacterial pathogenicity**

**MOLECULAR CHARACTERISTICS OF BRUCELLA ABORTUS MUTANTS  
GENERATED WITH EZ-TN5™ PMOD™-3 TRANSPOSON**

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**Background**

*Brucella abortus* is a well-known intracellular pathogen. Underlying mechanisms of the bacterial infection is very complicated to understand the pathogenesis even though most of clues have been solved. Therefore, prevention and control of the *B. abortus* infection is still problematic in animal and human. So, several methods including random mutation have been used to understand the mechanisms and find out the solution in control and prevention of the infection. Regardless the trials, the doors to be opened are still locked in understanding of the mechanism.

**Objectives**

To understand the underlying mechanism of *B. abortus* infection, mutants were generated with a transposon and function of the disrupted genes were revealed.

**Methods**

Mutants of *B. abortus* were generated by random insertion of a transposon, Ez-Tn5™ pMOD™-3 <R6K<sub>ori</sub>/MCS> into chromosome. Molecular characteristics of the mutants were investigated using PFGE, Southern blot and sequencing of the genes.

**Conclusions**

*B. abortus* mutants were generated and insertion of the transposon was confirmed by Southern blot analysis with the transposon as a probe after PFGE of chromosome. Both sides of insert in the chromosome were sequenced and the location of interrupted genes were identified in chromosome. Using sequencing information, twenty-eight genes were identified from the mutants by comparison with wild type of the bacteria. Also, function of the genes were revealed. This work was supported by NRF (No. 2014R1A2A2A01007291).

**FEMS-2498**

**Bacterial pathogenicity**

**CYTOKINE EXPRESSION PROFILE IN RAW 264.7 CELLS STIMULATED WITH BRUCELLA ABORTUS MUTANTS**

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**Background**

*Brucella abortus* have been known as an important intracellular, zoonotic pathogen. Intense immune responses are induced in animals by the bacterial infection. Cytokine has key roles in activation of innate immunity and acquired immunity. Precious roles of cellular components of *B. abortus* infection have been remaining to be solved even though involvements of immune cells in activation of acquired immunity are already known. On the basis of current understanding, production profiles of inflammation cytokines such as NO, IL-1 $\beta$ , IL-4, IL-12 and IFN- $\gamma$ , were compared with *Brucella* mutants. Moreover, it might be given a key to find immune system, pathological pathways, and mechanism.

**Objectives**

To understand the roles of cellular components of *B. abortus* in immune cells, production profiles of cytokines were investigated in a mouse macrophage cells, RAW264.7 cells after stimulation with *B. abortus* mutants.

**Methods**

RAW 264.7, murine macrophages, cells were stimulated with mutants of *B. abortus*. Culture supernatants were collected from RAW 264.7 cells after stimulation with *B. abortus* mutants at different time intervals. Amounts of cytokine such as NO, IL-1 $\beta$ , IL-4, IL-12 and IFN- $\gamma$ , were quantified with ELISA.

**Conclusions**

Inflammatory cytokines were produced from Raw 264.7, murine macrophage, cells after stimulation with the *B. abortus* mutants. After then, production levels of inflammatory cytokines such as NO, IL-1 $\beta$ , IL-4, IL-12 and IFN- $\gamma$ , were measured. The patterns of the cytokine productions will be presented. This work supported by NRF (No.2014R1A2A201007291) and IPET112012-03-1-HD020.

**FEMS-2991**

**Bacterial pathogenicity**

## **FUNCTIONAL INSIGHTS INTO THE ROLES OF AUTOTRANSPORTER ADHESINS IN BRUCELLA**

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### **Background**

*Brucella* pathogens proliferate within several eukaryotic cells. Cumulative evidence indicates that adhesion of *Brucella* to host cells is an important step to establish infection. We have shown that the unipolar monomeric autotransporter BmaC is involved in the adhesion of *Brucella suis* to host cells through binding to cell-associated fibronectin.

### **Objectives**

Genome analysis shows that the *B. suis* genome encodes two trimeric autotransporters (BtaE and BtaF) and two additional monomeric autotransporters (BmaA and BmaB). The aim of this work was to explore the roles of these proteins in the interaction of *Brucella* with the host.

### **Methods**

Clean deletion mutants were analyzed by in vitro binding assays and in vivo using the mouse model. Adhesin localization was analyzed by immunofluorescence.

### **Conclusions**

BtaE was involved in the binding of *B. suis* to hyaluronic acid and fibronectin while BtaF may contribute to the binding to several ligands. Both the  $\Delta btaE$  and  $\Delta btaF$  mutants showed a reduction in the attachment to epithelial cells and were attenuated in mice. Similar to BmaC, BtaE and BtaF were detected in a low proportion of bacteria but in all cases at one particular pole. A remarkable variability in the number of motifs of autotransporter orthologues from different *Brucella* species was observed.

Furthermore, while both trimeric autotransporters might be functional in all *Brucella* species, pseudogenization occurred in *bmaA* and *bmaB* orthologues. In particular, BmaA would not be functional in *Brucella abortus* while it is predicted to be functional in *B. suis* and *B. melitensis*. Thus, functional autotransporters may contribute to host preference.

**FEMS-1204**  
**(Human) microbiome**

**DISCOVERY OF A CONJUGATIVE MEGAPLASMID IN BIFIDOBACTERIUM BREVE**

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**Background**

*Bifidobacterium breve* is a common inhabitant of the infant gut and its presence has been correlated with particular health-promoting effects, such as enforcement of the intestinal barrier, activation/modulation of the host's immune response and protection against particular infections.

**Objectives**

Genome sequencing of *B. breve* JCM 7017 revealed the presence of the first verified bifidobacterial megaplasmid pMP7017 (190 kb of size). The objective of this study was to provide evidence of its horizontal transmission between strains of the same and/or different (bifido)bacterial species by means of a predicted conjugative machinery.

**Methods**

DNA sequencing was performed using Next Generation Sequencing platforms. The *in silico* predictions were performed using BLAST and PFAM databases as well as additional bioinformatics tools (e.g prodigal, tRNAscan, CRISPR and GCUA). Proof of conjugal transfer was obtained by demonstrating the presence of the megaplasmid using Pulsed Field Gel Electrophoresis in combination with S1 nuclease treatment.

**Conclusions**

*In silico* characterization of the megaplasmid revealed several genomic features supporting a stable establishment in its host, illustrated by predicted CRISPR-Cas functions that are known to protect the host against intrusion of foreign DNA. Interestingly, pMP7017 is also predicted to encode a conjugative DNA transfer apparatus and consistent with this notion we demonstrate conjugal transfer of pMP7017 to representative strains of *B. breve* and *B. longum* subsp. *longum*. We furthermore demonstrate the presence of a megaplasmid with homology to pMP7017 in three *B. longum* subsp. *longum* strains, indicating that similar elements are also naturally present in other bifidobacterial species, thereby supporting our proof of cross-species transfer.





**FEMS-1696**  
**(Human) microbiome**

**EXPLORING THE INFLUENCE OF CHEMOTACTIC PREFERENCES ON  
SUBSTRATE COMPETITION AND COMMUNITY FATE BY IN SILICO MODELING**

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**Background**

Competition for substrate is a common mode of microbial interaction in natural environments. Both, growth and motility phenotype influence competition, with the former having been studied well, while the latter has received less attention.

**Objectives**

To elucidate the impact of microbial motility on competition, we focus on microbial communities in which two strains populating a homogeneous environment compete for a substrate. Strains share identical growth phenotypes but differ in their chemotactic preference, either responding more sensitively to substrate or to a chemoattractant that is excreted by the cells themselves. By systematically varying chemotactic preferences of the competing strains, we assess the impact of each chemotactic preference on competition and on overall community fate.

**Methods**

Microbial communities are simulated using an individual-based modelling approach. Competing strains are initially homogeneously populating a two-dimensional environment. Microbial growth and motility including random movement and chemotaxis towards substrate and microbial cells, and substrate diffusion are simulated, and the resulting spatio-temporal microbial distribution patterns are observed and analyzed.

**Conclusions**

Results show that, depending on the combination of chemotactic preferences, microbial distributions remained homogeneous or transient or permanent spatial patterns emerged. The presence of a competing strain is able either to suppress or to induce aggregate formation, indicating more complex interactions beyond simple substrate competition. Strains being more attracted to self-excreted compounds tend to form aggregates of high densities where starvation conditions prevail, leading communities to become dominated by the competing strain. The model indicates a mechanism by which aggregate formation can evolve, even if it is initially disadvantageous.

**FEMS-3121**  
**(Human) microbiome**

## **INDIVIDUAL-BASED MODELLING OF THE IMPACT OF INITIAL EVENNESS ON THE PRESERVATION OF BIODIVERSITY FOR IN SILICO BACTERIAL COMMUNITIES**

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### **Background**

One of the most important aims in ecology is to identify and comprehend the mechanisms that sustain biodiversity - often critically important for ecosystem viability. Many theoretical models have shown that species in competition can coexist – thus maintaining the ecosystem's biodiversity - if ecological processes such as competition and mobility occur on a local scale [1]. This is also true in the case of communities with non-transitive competition between species, the classical example being the rock-paper-scissors game.

### **Objectives**

Initial community evenness has been shown to be a key factor in preserving ecosystem functional stability [2], but has not been accounted for in previous modelling studies. We formulate a model that allows initial community evenness to be varied in order to investigate the consequent impact on ecosystem biodiversity.

### **Methods**

We consider an ecosystem of four interacting bacterial species, and present a stochastic, spatial individual-based model simulating the ecosystem dynamics. Interactions take place on a two-dimensional lattice. Three processes are incorporated: reproduction, competition and mobility. In addition to variable initial evenness, multiple competition schemes are implemented, modelling various possible communities, resulting in diverse coexistence and extinction scenarios.

### **Conclusions**

Simulations show that long-term ecosystem behaviour is strongly dependent on initial evenness and competition structure. The ecosystem is generally unstable; higher initial evenness has a small stabilizing effect on ecosystem dynamics by extending the time until the first extinction.

[1] T. Reichenbach et al., Mobility promotes and jeopardizes biodiversity in rock-paper-scissors games. *Nature* (2007).

[2] L. Wittebolle et al., Initial community evenness favours functionality under selective stress. *Nature* (2009).

**FEMS-1409**  
**(Human) microbiome**

**MECHANISM BASED PHARMACODYNAMIC MODELING OF GROWTH  
INHIBITION IN BACTERIA**

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**Background**

Antibiotic activity is assessed in vitro by quantifying the reduction of growth of a bacterial culture. Growth can be described as change in cell number on population level. On the single-cell level, physiological properties like cell size before division or translational capacity vary with growth rates: Cells adapt to their environment. Typical drug-effect models link population growth directly to the exposure of antibiotics. They rarely account for known mechanisms of action of the drug - which are particularly relevant for the analysis of synergistic or antagonistic effects of drug combinations.

**Objectives**

Development of a pharmacodynamic model which allows for mechanistic integration of antimicrobial effects on the cellular level to predict bacterial growth.

**Methods**

Control experiments without drug resulted in baseline values for population growth. An established single cell model predicted cell level parameters from this growth rate. Induction of cell killing and growth inhibition were linked to known drug characteristics (minimal bactericidal / inhibitory concentration). A transit compartment cell-cycle model integrated time dependent cellular adaptation processes. Two bacterial subpopulations were considered (resting and growing). We validated our model for predicting time-kill curves with *E. coli* exposed to tetracycline. Furthermore we predicted septation dynamics of *B. subtilis* during a shiftdown from exponential to stationary phase.

**Conclusions**

Since both scenarios show good agreement between predicted and experimental data, these promising results are a first step to mechanistically model bacterial growth during exposure to multiple antibiotics.

**FEMS-0853**  
**(Human) microbiome**

## **INFLUENCE OF ACTIVE SITE GROOVE VOLUME ON DD-CARBOXYPEPTIDASE ACTIVITY OF *E. COLI* DACD**

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### **Background**

*E. coli* DacD is a DD-carboxypeptidase, having much weaker DD-CPase activity than PBP5. The reason for such a weak DD-CPase activity of DacD and its importance within an *E. coli* cell is unknown.

### **Objectives**

Deciphering the role of active-site groove volume (AGV) on DD-CPase activity of *E. coli* DacD.

### **Methods**

Here, we generated the 3D model of DacD using homology modeling and analysed the binding of specific substrates within AGV. Interestingly, the AGV of DacD is about one-third of PBP5 and such a low AGV might reduce the catalytic efficiency of DacD. To validate the result *in silico*, we mutated the secondary amino-acid residues around the DacD active-site by incorporating several transitional and transversional mutations and observed for the changes developed in the AGV. Such mutations brought a 70% increase in the mutant DacD AGV. PBP5 has higher AGV that can be correlated with DD-CPase activity. In addition, we superimposed PBP5 active-site onto mutated DacD and observed a similar geometric arrangement. Furthermore, the pentapeptide-binding affinity of the mutant DacD was increased as revealed by molecular docking analyses. Higher binding affinity of the mutant DacD towards peptidoglycan mimetic pentapeptide substrate possibly signifies a greater DD-CPase activity.

### **Conclusions**

Active-site groove volume may act as a determinant for DD-CPase activity and the reduced DD-CPase activity of DacD is possibly due to its inadequate active-site groove volume.



**FEMS-3082**  
**(Human) microbiome**

**TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL REGULATION OF PST2  
OPERON EXPRESSION IN VIBRIO CHOLERAЕ 01**

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**Background**

The most abundant periplasmic protein of *Vibrio cholerae* grown under inorganic phosphate (Pi) limitation is the high affinity Pi transporter, PstS, of the Pst2 system. It is encoded by *pstS* of the *pst2* operon, *pstSABC*, member of *V. cholerae*'s Pho regulon. Little is known about the regulation of *pst2* expression and the non-stoichiometric production of its genes products.

**Objectives**

To elucidate the regulatory mechanisms behind *pst2* expression in *V. cholerae*.

**Methods**

Bioinformatics analysis: Clustal Omega for protein sequences alignment, Mfold for the determination of RNA secondary structures and the corresponding free energy values and MEME/MAST to search for putative Pho boxes. Experimental procedures: SDS-PAGE and MS *ESI-Q-ToF Micro* for protein analysis and identification and promoter-*lacZ* fusion, RNA extraction, cDNA synthesis, endpoint-PCR and quantitative Real-Time PCR for the expression analysis.

**Conclusions**

Based on the bioinformatics analysis we proposed new start codons for *pstS* and *pstC*, a regulatory region upstream *pstS* containing potential Pho boxes and a *pstS-pstC* intergenic region, distinct from those predicted. Moreover, sequences able to form stem-loop structures followed by potential RNase E-cleavage sites were detected in the intergenic regions *pstA-pstB* and *pstS-pstC*. The experimental analyses demonstrated that *pst2* is transcribed under Pi limitation in a PhoB-dependent manner as a full-length mRNA that is processed into minor transcripts of distinct stabilities. The most stable is the *pstS*-encoding mRNA, followed by *pstB*, *pstA* and *pstC* specific transcripts. The abundance of PstS relative to the other components of Pst2 system in *V. cholerae* seems to correlate to the higher stability of its transcript.





**FEMS-1863**  
**(Human) microbiome**

**MICROBE ATLAS: A GLOBAL INTEGRATED MAP OF MICROBIAL DIVERSITY**

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**Background**

The world's microbial diversity is vast and the majority of microbes do not readily grow in culture, complicating the analysis and interpretation of this diversity. In addition, the lack of a unified and consistent annotation of the world's microbial diversity has so far impeded a comprehensive comparative analysis.

**Objectives**

The "Microbe Atlas", a global metaresource of all publicly available environmental marker gene sequence data, which will be available as a web resource. A dataset consisting of 212'000 samples with more than 3 billion identified small subunit ribosomal RNA sequences. This resource will enable a better understanding of what are the typical and atypical microbial taxa in samples, by establishing compositional baselines in terms of microbes that are often found in similar environments.

**Methods**

An analysis of all publicly available environmental marker gene sequence data.

**Conclusions**

This resource should become an essential tool for microbial biologists wishing to compare their samples to previously sequenced microbial communities, and identify both the typical as well as atypical microbial taxa in their samples.

**FEMS-1143**  
**(Human) microbiome**

**LACCASE FROM PATHOGENIC GUT BACTERIA FACILITATE BIOTRANSFORMATION OF NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)**

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**Background**

Non-steroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of pain, inflammation, cardiovascular diseases, arthritis and cancer by inhibiting cell proliferation. Laccases (EC 1.10.3.2) are multicopper oxidases (MCO), recently reported from *Escherichia coli*, *Salmonella enterica*, *Camylobacter jejuni*, *Pseudomonas aeruginosa* with role in copper homeostasis, iron acquisition, manganese oxidation and in pathogenesis.

**Objectives**

To find out novel laccase from different serotypes of *Yersinia enterocolitica* and clone it into a suitable expression vector for the biotransformation of NSAIDs.

**Methods**

Amplified laccase gene (*yack*) from different serotypes of *Y. enterocolitica* was cloned in pTZ57R/T vector using TA cloning method. The positive clones were sequenced to confirm *yack* and construct a phylogenetic tree using Mega 5.0. Further, *yack* was subcloned into the desired restriction sites of pET28a expression vector and transformed into *E. coli* BL21 (DE3) expression host. The recombinant protein was refolded and purified using His-spin protein miniprep kit, for the biotransformation of aspirin and diclofenac (NSAIDs), which validates our *in silico* studies.

**Conclusions**

Laccase from different strains of *Y. enterocolitica* can be used as a taxonomic tool as it showed serotype specific clades. The HPLC analysis of laccase treated diclofenac and aspirin showed complete biotransformation after 24h of incubation. The FTIR results suggested a change in C-C and C-N bonds. Laccase transform NSAIDs and may have a significant role in the colonization of laccase positive gut microflora.

**FEMS-1411**  
**(Human) microbiome**

## **RELATIONSHIPS BETWEEN MICROBIAL GENE CONTENT, PHYLOGENETIC DIVERGENCE AND ENVIRONMENTAL PREFERENCES**

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### **Background**

Environmental characteristics impose some particular constraints to the species living in any given habitat. In many instances, prokaryotic taxa show environmental preferences that shape the diversity found in diverse different environments. It is reasonable to expect that these habitat preferences have a reflection on the metabolism of the prokaryotic organisms. All these metabolic choices require particular sets of genes, and, therefore, gene content is determined to some extent by environmental preferences. But it is unclear to what extent is this relationship determinant, or how extensive is the metabolic rearrangement leading to different environmental adaptations.

On the other hand, it seems obvious that genomic content is determined highly by phylogenetic proximity. The extent to which each of the two processes modulates the genomes has not been quantified.

### **Objectives**

We present a comprehensive analysis of the relationships between phylogeny, metabolism and environmental similarity in the prokaryotic world. We aim to quantify the influences of phylogenetic descent and environmental preferences in genomic content, to answer the question of how much it is shaped by each of the contributions.

### **Methods**

We have calculated measures of phylogenetic, genomic and environmental distances between pairs of prokaryotic taxa using their 16S distances, common gene content and environmental preferences.

### **Conclusions**

The results indicate a strong influence of the phylogeny in the genomic content, although the influence of the environment is notable for some particular metabolic

classes. This allowed to discover sets of genes that can act as environmental markers and predictors of the possible habitats for new species.

**FEMS-3124**  
**(Human) microbiome**

**DISCOVERY OF PUMILARIN, A NOVEL HEAD-TO-TAIL CYCLIZED PEPTIDE, BY BAGEL3**

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**Background**

In the post-genomic era the amount of (public) genomic data is ever increasing. Bacteriocin mining tools can be used to extract valuable information from this data regarding the presence of post-translationally modified peptides.

**Objectives**

As a proof of principle we mined all publicly available bacterial genomes using Bagel3. For simplicity we limited the results to head-to-tail cyclized peptides only. We found a large variety of these (predicted) circular peptides in the *Bacillus* genus. Based on the mining results we screened several *Bacillus pumilus* strains for the production of novel (head-to-tail) bacteriocins.

**Methods**

This yielded the production of a novel bacteriocin that we call pumilarin. The overall homology with AS-48 is about 50%. We purified pumilarin using protocols that are based on AS-48 purification methods.

**Conclusions**

Next we analysed its structure using mass-spectrometry and found that its mass was in accordance with its circular structure. After fragmentation masses could be observed that could be explained only by N- to C-terminal linkage of the peptide. The activity spectrum of pumilarin was benchmarked against the prototype head-to-tail cyclized bacteriocin AS-48 and found to show some interesting differences in spectrum.

**FEMS-3148**  
**(Human) microbiome**

**MATHEMATICAL MODELLING OF BACTERIAL CROSS-FEEDING:  
APPLICATION IN THE HUMAN INTESTINAL TRACT**

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**Background**

In nature bacteria are rarely found in isolation, rather they reside in close association and are commonly found within biofilms. Bacteria that develop alternative feeding mechanisms, such as cross-feeding, have a much greater chance of survival in a competitive environment. In the human intestinal tract, the many types of cross-feeding include the use of oligosaccharides and/or monosaccharides resulting from extracellular degradation of polysaccharides by a competing bacterium, the use of such molecules along with metabolites produced by other bacteria and solely using metabolites produced closely associated bacteria.

**Objectives**

We aimed to develop models to describe the three aforementioned types of microbial cross-feeding and use those models to understand the cross-feeding phenomenon.

**Methods**

We developed a system of mechanistic differential equations to describe the different types of bacterial cross-feeding. These models were fit to multi-response data found in the literature using nonlinear regression. The multi-response data included bacterial growth (*Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Roseburia*), substrate degradation (inulin, oligofructose, fructose, and metabolites), and metabolite concentrations (succinate, acetate, lactate and butyrate). To ensure that the estimated model parameters were independent of scale, we minimized the diagonal of the matrix  $ZTZ$ , where  $Z$  is the residual matrix formed from the data and model fit.

**Conclusions**

Our results suggest that microbial cross-feeding follows second-order chemical kinetics even when the ratios of two substrates are not one-to-one.

**FEMS-0855**  
**(Human) microbiome**

## **COMPARATIVE GENOMIC ANALYSIS REVEALS SUBTELOMERIC GENE DUPLICATIONS AND METABOLIC POTENTIAL OF THE KLUYVEROMYCES GENUS**

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### **Background**

The yeasts *Kluyveromyces lactis* and *Kluyveromyces marxianus* are the best-studied species of the *Kluyveromyces* genus. *K. lactis* has been adopted as a model for non-*Saccharomyces* yeasts whereas *K. marxianus* is more widely used for a range of different industrial applications because of relevant phenotypic traits. A *K. lactis* genome sequence has been available for some time but only recently genome sequences for a number of different *K. marxianus* strains have been generated.

### **Objectives**

Our aim to understand the genetic basis of the metabolic and physiological differences between *Kluyveromyces* and *Saccharomyces* and within the *Kluyveromyces* genus.

### **Methods**

We compared the proteomes of *K. marxianus*, *K. lactis* and *S. cerevisiae* by protein clustering using OrthoMCL. We found 4,119 common clusters (in all 3 yeasts) and 596 exclusively shared by the two *Kluyveromyces* species. More detailed bioinformatics as well as phenotypic assays were carried out to verify the findings.

### **Conclusions**

Some key differences arise in sub-telomeric regions, where the same genes can be found duplicated at each end of the chromosome or at the ends of a separate chromosomes. For example, the genes responsible for lactose assimilation are duplicated in the subtelomeric regions of *K. marxianus* but not in *K. lactis*. Indeed, detailed comparison this duplicated region in different *K. marxianus* strains identifies variation that may account for some of the phenotypic differences in lactose assimilation between strains. Other differences that give rise to phenotypic variation



between species have also been verified and new metabolic pathways identified in *K. marxianus*.

**FEMS-0584**  
**(Human) microbiome**

**COMPARATIVE GENOMIC ANALYSIS OF NINE SPHINGOBIUM STRAINS:  
INSIGHTS INTO THEIR EVOLUTION AND HEXACHLOROCYCLOHEXANE (HCH)  
DEGRADATION PATHWAYS**

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**Background**

Species belonging to the genus *Sphingobium* are well known for their ability to degrade hexachlorocyclohexane (HCH) isomers due to the presence of *lin* pathway genes. However, the establishment of the *lin* pathway in sphingomonads under HCH pressure is still unknown.

**Objectives**

Here, the nine *Sphingobium* spp. (strains LL03, DS20, IP26, HDIPO4, P25 and RL3, B90A, UT26S and *Sphingobium* sp. SYK6) were compared in order to reveal the mode of acquisition of *lin* genes.

**Methods**

Genomes of *Sphingobium* spp. were assembled using ABySS1.3.3 followed by their validation with bwa0.5.9. Phylogeny was constructed with 16S rRNA, single copy genes, ANI and tetranucleotide frequency. Further, draft genomes were annotated using Glimmer-3 at RAST4.0 server. Orthologs were identified using CD-HIT and all versus all BLAST. Functional annotation was performed using KAAS and gene families were predicted using Minpath. dN/dS and dS for each gene pair was calculated using Hyphy2.1.2.

**Conclusions**

The phylogenetic analysis using genomic data clustered efficient HCH degraders in a closely related group comprising of UT26S, B90A, HDIPO4 and IP26, where HDIPO4 and IP26 were classified as subspecies with ANI value >98%. Total gene content shared among all nine strains was ~10% which increased to ~25% in eight HCH-associated strains. Additionally, an inter-genus plasmid pool between genus *Sphingobium* and *Sphingomonas* was observed. Further, the differences in *lin* gene sequences, copy number and arrangement with respect to IS6100 revealed possible

evolutionary acquisition mechanisms for this pathway. This study also reflected that HDIPO4, IP26 and B90A are better suited organisms for HCH bioremediation.

**FEMS-2976**

**Environmental microbiology - 2**

**DIVERSITY OF SURFACE-ASSOCIATED BACTERIA AND ANTIBACTERIAL ACTIVITY OF THE MARINE SPONGES COLLECTED FROM MARINE AREAS OF TURKEY**

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**Background**

Increasing resistance problem of human pathogenic bacteria against commercial antibiotic derivatives has become important problem of all over the world.

**Objectives**

Anti-bacterial activity of the methanolic extracts of the marine sponges against pathogenic bacteria and diversities of sponge-associated and free-living bacteria were investigated.

**Methods**

Taxonomic designations of the sponges were carried out using histological sample preparation technique. The samples were extracted using cold methanol extraction technique. The agar disc diffusion method was used to determine the anti-bacterial activity of the sponge extracts. The pure bacterial strains isolated from the sponges were identified using the automated micro identification system VITEK2 Compact30.

**Conclusions**

The methanolic extracts of all tested sponges displayed positive antibacterial activity at varying levels against pathogenic bacteria. Antibacterial effectiveness rate was found higher in the samples which were collected from the Aegean Sea than the Sea of Marmara. The community profiles of the sponge associated bacteria were found different from free-living bacteria. Though similar bacteria species were found in all sponge samples, the predominant bacterial community profiles were different. Of the 325 unit bacterial strains, isolated from the Aegean Sea sponges, 86% belonged to phylum Proteobacteria. 82% of 102 bacterial strains, isolated from the sponges of the Sea of Marmara, were Proteobacteria. The study resulted in increasing data to understand antibacterial activity of the marine sponges regarding diversities of the sponge/sponge-associated bacteria according to their exposure local environmental conditions in the in the different marine areas.

## EFFECT OF TEMPERATURE ON BACTERIOPHAGE-MEDIATED BACTERIAL LYSIS EFFICIENCY

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### Background

Bacteriophages (phages) are viruses that specifically target bacteria. This property makes them useful agents for the control of pathogenic bacteria. Despite their great potential there is great uncertainty about the environmental parameters affecting phage-mediated lysis efficiency.

### Objectives

In this study, temperature was investigated as a key environmental factor.

### Methods

Three distinct phages specific for *E.coli* were isolated from a single brook water sample. Lysis efficiency was monitored using the double agar overlay method and optical absorbance measurements. Plaque sizes and numbers varied dramatically depending on whether phage-host mixtures were incubated at 20°C, 30°C or 37°C with bigger and more plaques being visible at lower temperatures. Interestingly plaque formation was invisible at the optimum temperature of the host (37°C). Plaque numbers and sizes were found to additionally depend on the temperature history of the bacterial host prior to mixing with the phage. This applied both to the growth temperature of the *E. coli* (with higher plaque numbers and sizes when cells were grown at lower temperature) and short term temperature exposure of fully grown cells. Exposure of *E. coli* to sublethal heat stress resulted in a phage resistant phenotype.

### Conclusions

These outcomes suggest that many phage-host interactions might be highly temperature-sensitive and phages seem to prefer host cells that were subjected to temperature conditions similar to the ones in the environment where the phages were isolated from. Phages-host interactions should be well characterized for their temperature preference and probably other environmental parameters prior to biocontrol and biosanitization applications to obtain optimal performance.

**FEMS-1728**

**Environmental microbiology - 2**

**CHANGES IN MEMBRANE SUBPROTEOME IN VIBRIO HARVEYI DURING THE ADOPTION OF VIABLE BUT NONCULTURABLE STATE BY EFFECT OF STARVATION AND VISIBLE LIGHT**

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**Background**

*Vibrio harveyi* is a pathogen bacterium for a wide range of marine animals that can be isolated as a planktonic form. The behaviour of *Vibrio* spp. in natural environments is conditioned, among others, by temperature, light, salinity or nutrient scarcity.

**Objectives**

The aim of this study was to evaluate the physiological and proteomic changes that take place during *V. harveyi* survival in seawater under exposure to visible light.

**Methods**

For this purpose, populations of *V. harveyi* were maintained in sterile natural seawater at 20°C and under illumination for at least 21 days. Along experiments, total, active and culturable populations were enumerated and the outer membrane subproteome was analyzed.

**Conclusions**

Experimental conditions had a negative effect upon culturability of *V. harveyi*, inducing its entry into the viable but nonculturable (VBNC) state after 6-7 days of exposure, and provoked changes in the membrane subproteome composition.

Along the period of study, some outer membrane proteins remained unchanged (e.g. OmpW), while a set of proteins increased (i.e. lipoproteins and agglutination proteins) or became detectable under tested conditions (in this last group the transport-related proteins are especially relevant). So, all these proteins could be likely essential for sustaining the *V. harveyi* viability under stress conditions. In contrast, several proteins and especially those related to chemotaxis processes decreased or were undetected along survival.

Therefore, in response to starvation and exposition to visible light *V. harveyi* experiments an adaptation process which includes the entry into the VBNC state and the membrane subproteome reorganization.



**FEMS-0582**

**Environmental microbiology - 2**

## **BIOTECHNOLOGICAL POTENTIAL OF BACTERIA ISOLATED FROM MARINE SPONGES**

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### **Background**

Marine sponge contains a great number of microorganisms present in their tissues. These ones perform symbiotic associations with their hosts, and are capable of producing secondary metabolites with various biotechnological properties.

### **Objectives**

Aiming to know more about microbial diversity present in aquatic environments and get bacterial species with potential application in biotechnology

### **Methods**

three species of marine sponges (*Hymeniacidon heliophila*, *Amphimedon viridis* and *Aplysina fulva*) were collected in two beaches of Rio de Janeiro, Brazil;. 38 bacterial isolates from these sponges were tested for the production of amylase, cellulase and caseinase using standard methodology.

### **Conclusions**

Of these, 19 showed enzymatic activity, 5 for amylase, 4 to cellulase and 16 for production of caseinase. Of all isolates tested only one colony was able to degrade the three substrates. The tests of biosurfactants' production, showed that 17 colonies were able to emulsify diesel. Our results show that the species of marine sponges studied are a great substrate for prospecting microorganisms with potential biotechnological applications



**FEMS-2216**

**Environmental microbiology - 2**

## **TOXICITY OF TERRESTRIAL CYANOBACTERIAL STRAINS ORIGINATED FROM FORESTS OF THE SERBIAN MOUNTAINS**

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### **Background**

As one of the oldest and highly diverse group of photosynthetic prokaryotic microorganisms, cyanobacteria are known as potent producers of various bioactive compounds with toxic effects.

### **Objectives**

Toxicity of five forest terrestrial, filamentous cyanobacterial strains belonging to *Oscillatoria* and *Nostoc* genera was investigated.

### **Methods**

The toxicity of intracellular extracts of cyanobacterial strains was tested in *Artemia salina* bioassay. The LC<sub>50</sub> values were recorded in all tested strains after 24 h (*Nostoc* genera) and 48 h (*Oscillatoria* genera). In the case of three *Oscillatoria* strains, a dose-dependent response in mortality that increased over time was observed after 24 h (1%-8%) and 48 h (72%-98%) of exposure. However, for the intracellular extracts of two strains, *Nostoc* T7 and *Nostoc* M2, toxicity was higher after exposure time of 24 h (70% and 97%) and 48 h (93% and 98%). According to the LC<sub>50</sub> values, cyanobacterial strain *Nostoc* M2 showed to be the most toxic strain with LC<sub>50</sub> value 0,59 mg/ml, followed by strain *Nostoc* T7 (LC<sub>50</sub>=1,25 mg/ml). Concerning *Oscillatoria* genera, the recorded LC<sub>50</sub> values were 3,49 mg/ml (*Oscillatoria* M1), 4,55 mg/ml (*Oscillatoria* M2) and 5,62 mg/ml (*Oscillatoria* T18).

### **Conclusions**

The results presented in this paper indicate that toxin production is strain-specific property, whereby intracellular content of three strains belonging to *Oscillatoria* genera was found to be less toxic to *A.salina* compared to the strains belonging to *Nostoc* genera. An additional objective which should be done is analysis and identification of toxic compounds present in the extracts responsible for observed toxicity.

**FEMS-0518**

**Environmental microbiology - 2**

**STUDIES ON PRODUCTION AND PURIFICATION OF BIOFLOCCULANT BY A BACTERIUM SPECIES ISOLATED FROM ADETI STREAM IN ILESA, OSUN STATE**

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**Background**

Water clarification methods like flocculation, coagulation and sedimentation are often inappropriate because of the high cost and scarcity of chemical coagulants. This study was designed to provide a safe, cheaper and biodegradable flocculating agent.

**Objectives**

The objectives include isolation of bacterial isolate with flocculating properties from wastewaters and soil samples ; selection of a bacterium with the highest bioflocculant-producing potential; determination of the time course of bioflocculant production; and purification of the bioflocculant.

**Methods**

Wastewater and soil samples were collected from Ilesa and plated on yeast extract, peptone and glucose (YPG) agar. Plates were incubated at 25°C for 24 h. Colonies with mucoid appearance were selected. Pure isolates were stored on sterile YPG agar slants. Bioflocculant production was carried out by growing the organism in YPG medium at 25°C and centrifuged at 5000 rpm for 30 min. Flocculant activity was measured by using a suspension of kaolin clay. Time-course of bioflocculant production was determined by monitoring pH, cell growth and flocculating activity. Bioflocculant was purified by precipitation and gel-filtration chromatography.

**Conclusions**

The highest bioflocculant –producing isolate was identified as *Pseudomonas extremaustralis* ABL19. Maximum bioflocculant production was observed at the 66<sup>th</sup> h. The optimal bioflocculant activity of 94.4% was achieved with glucose and peptone in the medium. The molecular weight was estimated to be 60.8kDa. The bioflocculant was effective at 80°C and alkaline pH.



**FEMS-1470**

**Environmental microbiology - 2**

## **CHARACTERIZATION OF BIOACTIVE COMPOUNDS PRODUCED BY SOIL STREPTOMYCES SPP.**

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### **Background**

In general special properties of different geographical area could be considered as best sources of bioactive compounds, therefore investigation on these fields might be increased treatment of patients and reduce the rate of occurrence of antibiotic-resistant bacteria.

### **Objectives**

Major purpose of this study was isolation of antimicrobial metabolite producing *Streptomyces* spp. from soil samples and identification the structure of their antimicrobial metabolites.

### **Methods**

To perform the present study, 70 strains of *Streptomyces* spp. were isolated from soil samples and evaluated for producing of their antimicrobial products against some pathogenic microorganisms. Then, the antimicrobial producing strains were identified using phenotypic and genotypic methods. The best growth phase of production was determined and finally structures of antimicrobial compounds were assessed by <sup>13</sup>CNM, <sup>1</sup>HNMR and FT-IR methods. Regarding to determination of possible structures, all data obtained from <sup>13</sup>C NMR, <sup>1</sup>H NMR and FT-IR were analysis by NCBI PubChem Structure Search program.

### **Conclusions**

Out of 70 strains of indigenous *Streptomyces* spp., three strains had potent activity for production of antimicrobial metabolites. The isolates recognized as *Streptomyces griseus*, *Streptomyces phaeochromogenes* and *Streptomyces olivoviridis*. Metabolites

showed antimicrobial activity against *Escherichia coli*, *Salmonella typhi*, *klebsiella pneumonia*, *shigella dysenteriae*, *Bacillus cereus* and *Staphylococcus aureus*. The best growth phase for antimicrobial metabolite production was stationary phase, therefore based on these data all the metabolites recognized as secondary metabolites. Regarding to determination of possible structures, all data obtained from <sup>13</sup>C NMR, <sup>1</sup>H NMR and FT-IR were analysis by NCBI PubChem Structure Search program and resulted suggestion three possible structures of bioactive compounds.

**FEMS-2707**

**Environmental microbiology - 2**

## **ISOLATION AND CONSERVATION OF FLUORESCENT PSEUDOMONADS STRAINS FROM RHIZOSPHERE OF WHEAT**

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### **Background**

Plant Growth Promoting Rhizobacteria (PGPR) meets those of inhomogeneous rhizobacteria groups which are patented to increase and promote plant growth. Pseudomonas bacteria is one of the most important PGPR bacteria which can increase yield, growth and health of the plant through direct and indirect mechanisms.

### **Objectives**

In the present study, fluorescent pseudomonads isolated from Savadkooh's soils have been investigated with respect to their biochemical tests and physiological characteristics.

### **Methods**

In the first experiment, 30 soil samples were collected from the rooting zone of wheat at farmer's field in Savadkooh, Mazandaran province, Iran. The soil samples were serially diluted up to 6–7, plated on King's B (KB) agar medium and incubated at  $28 \pm 2^\circ\text{C}$  for 48 h. Distinct colonies showing fluorescence under UV light were picked and streaked on KB agar medium to check the purity. In the second experiment, for the identification of fluorescent pseudomonads, certain biochemical tests and physiological characteristics were conducted according to Bergey's Manual for determinative bacteriology.

### **Conclusions**

The results showed that 17 pseudomonas strains were detected based on yellow, green and blue pigments by viewing under UV light. Population of bacteria were  $2.71 \times 10^5 - 6.43 \times 10^7$  cell per gram of rhizosphere soil. Based on results of biochemical tests and physiological characteristics we were detected three species, *P. putida* > *P.*

*fluorescens* >> *P. aeruginosa*. Dominate species of fluorescent pseudomonads in rhizosphere of wheat was putida.

**FEMS-2736**

**Environmental microbiology - 2**

**SEASONAL DYNAMICS OF SOIL MICROBIAL COMMUNITIES UNDER  
DOMINANT UNDERSTORY VEGETATION IN SPRUCE SWAMP FOREST**

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**Background**

Spruce swamp forests (SSF) are exceptional ecosystems which are considered to be important sources of greenhouse gases (GHG). Understorey vegetation dominates *Eriophorum vaginatum* and *Vaccinium myrtillus* which create patchy environment in SSF may mitigate GHG emissions. Presence of *Eriophorum* or *Vaccinium* can affect GHG emissions through the quality and quantity of its litter and root exudates. Their input directly affects microbial community and its decomposition capabilities.

**Objectives**

Our two main objectives were (i) to describe the seasonal dynamics of bacterial and archaeal communities in spruce swamp forests under the two dominant understorey vegetations (*Eriophorum* and *Vaccinium*) and (ii) to evaluate the effect of dominant vegetation on activity of soil enzymes.

**Methods**

To characterize microbial community we sequenced variable region V4 of 16SrDNA using Illumina MiSeq platform. OTU-picking and taxonomic assignment was performed using the QIIME 1.8.0 bioinformatics pipeline. Hydrolytic enzymes were analyzed by fluorometric method using 4-methylumbelliferone (MUB) labeled substrates.

**Conclusions**

Methanogenes (Methanomicrobia, Methanobacteria) dominated archaeal community followed by Thermoplasmata, MBGA and MCG (Fig. 1). Relative abundance of methanogens was significantly higher in *Spagnum* and *Eriophorum* than in *Vaccinium* sites. *Eriophorum* and *Vaccinium* sites were enriched by Proteobacteria in comparison to *Shagnum* sites (Fig. 2). On the other hand *Sphagnum* control site had higher relative amount of anaerobic bacteria (Firmicutes-Clostridia and Chloroflexi). Hydrolytic enzymes were dominated by phosphatase activity showing probably P



limitation which was highest on Sphagnum control site.

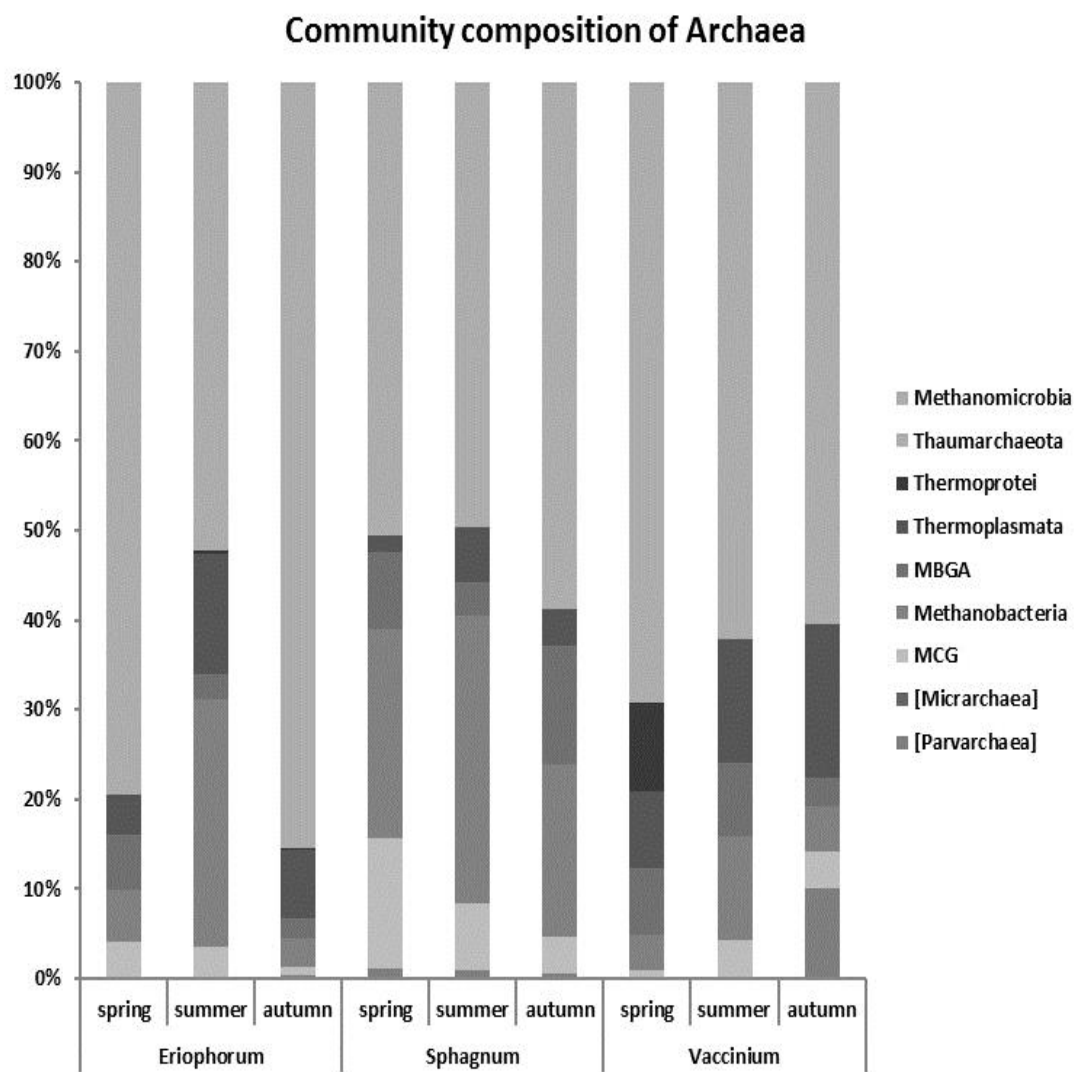


Fig. 1. Seasonal dynamics of archaeal community

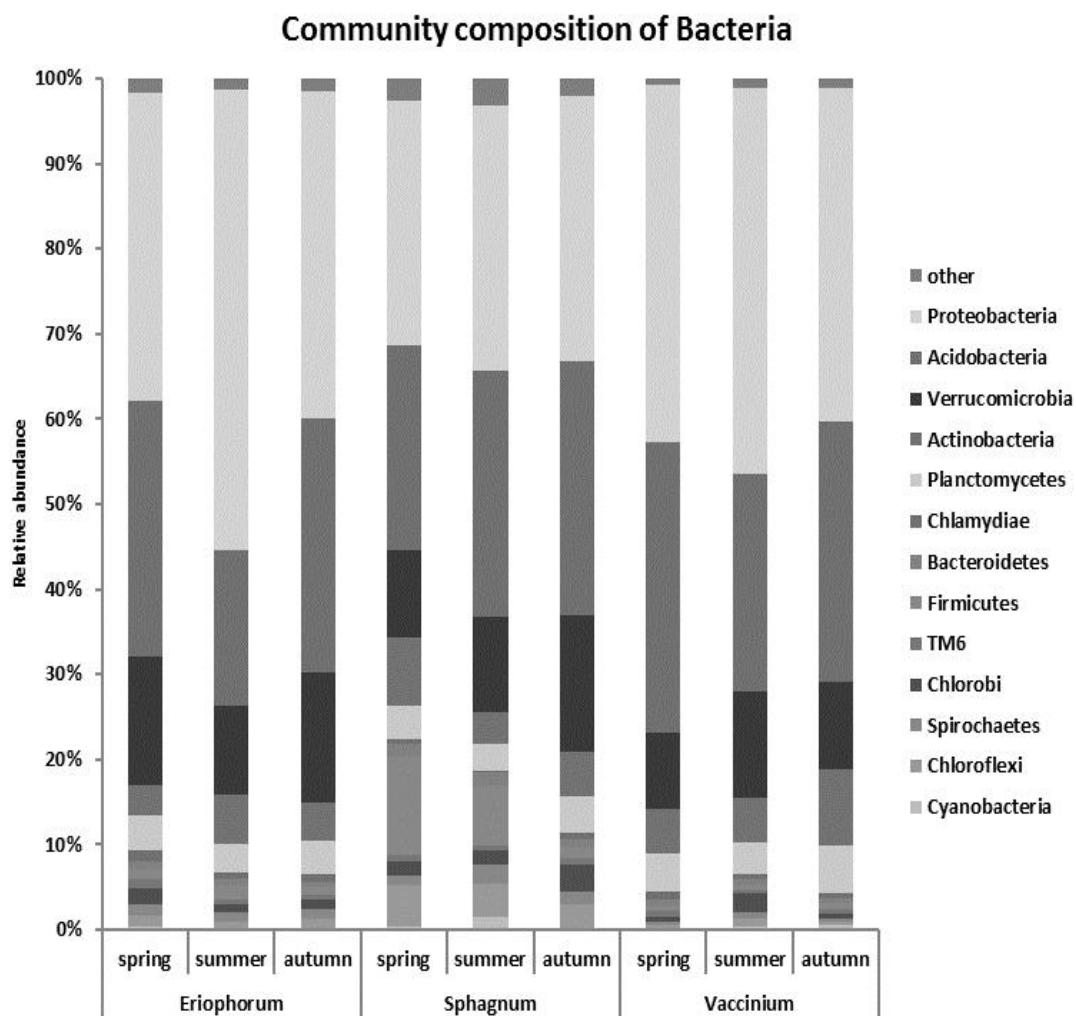


Fig. 2. Seasonal dynamics of bacterial community

**FEMS-3015**

**Environmental microbiology - 2**

**ANAEROBIC OXIDATION OF METHANE IN SURFACE SEDIMENTS OF MARINE LAKE GREVELINGEN, NORTH SEA**

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**Background**

Sulphate reducing anaerobic oxidation of methane (AOM) is a well-established phenomenon occurring in deep marine environments (~ below 100 m). However, relatively shallow anaerobic sediments containing methane and sulphate may harbour anaerobic methanotrophic microorganisms (ANME). Marine Lake Grevelingen is a former estuary with a water depth of ~ 40 m and salinity of 31 g/kg, which is separated from the North Sea by a dam. High rates of degradation and deposition of organic matter have resulted in methane rich anoxic sediments which combined with sulphate from sea water renders the site a potential niche for AOM.

**Objectives**

The main aim of this research was to explore whether AOM occurs in the surface sediment of Marine Lake Grevelingen.

**Methods**

Pore water chemical analysis, serum bottle incubation for activity tests, and microbial analysis by fluorescence *in situ* hybridisation (FISH) were conducted.

**Conclusions**

AOM was evident at depths of 5 to 15 cm in the sediment with a steep decline in CH<sub>4</sub> concentrations from ~5 mM at 20 cm depth to negligible at the sediment surface; concurrently, sulphide concentrations increased to 5 mM. *In vitro* incubations with CH<sub>4</sub> and SO<sub>4</sub><sup>2-</sup> showed sulphide production coupled to the consumption of sulphate at approximately equimolar ratios at 150 days. In contrast, sulphate and sulphide concentrations remained constant in biotic and abiotic control incubations. ANME and

archaeal cells were observed by FISH, which could be linked to AOM activity. Using three independent approaches, this study, for the first time provides evidence for the occurrence of AOM in sediments of Marine Lake Grevelingen.

**FEMS-2387**

**Environmental microbiology - 2**

**EXOPOLYSACCHARIDE PRODUCTION BY RHIZOBIUM ETLI STRAIN ZD13, A HEAVY METAL-RESISTANT SOIL ISOLATE**

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**Background**

Exopolysaccharide (EPS) production functions as a protective strategy which favors the survival of microbes under unfavorable environmental conditions, e.g., heavy metal stress. Rhizobia are Gram-negative, rod-shaped bacteria known for EPS production. Some EPS, due to the presence of negatively charged functional groups, may act as heavy metal-binding agents.

**Objectives**

Studies on the EPS produced by *Rhizobium etli* strain ZD13 (RheZD13), an isolate from a postindustrial soil of the Upper Silesia (southern Poland).

**Methods**

RheZD13 was grown in 4 liters of a culture medium under aerobic conditions (120 h, 30°C, 120 rpm). Bacteria were pelleted by centrifugation (60 min, 4°C, 11806 x g) and the supernatant was sterile-filtered (Ø 0.22 µm) and treated with cold (-20°C) ethanol to precipitate the crude EPS. The latter was dialyzed and analyzed by GC and GC-MS.

**Conclusions**

4.627 g of RheZD13 EPS were obtained. The EPS contained glucose and galactose (in a molar ratio 3:1) and pyruvic acid that was 4,6-linked to galactose. The acidic nature and the resulting multiplicity of potential binding sites for positively charged particles suggested that RheZD13 EPS may act as a biosorbent for heavy metals.

**MICROBIOLOGICAL AND PHYSICAL-CHEMICAL CHARACTERIZATION OF  
SLUDGE GENERATED FROM WATER PURIFICATION PROCESS OF HUMAN  
WATER CONSUMPTION IN BRAZIL**

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Water Treatment Plants (WTP) generate waste as residual decanters sludge due the production of potable water. The objective of this study was characterize microbiologically the sludge from three samples from the WTP of Londrina, Brazil, through microbiological indicators – as *Enterococcus* spp, sulphite-reducing *Clostridium* and pathogen *Salmonella* spp. The ‘multiple tubes’ technique was used to isolated bacteria. For physical-chemical analyzis parameters it was used Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), turbidity, pH and apparent color (according to APHA, AWWA and WEF-2005). The results showed that in the sample 1, the most probable number (MPN) of microorganism per 100mL was 2,1x10<sup>4</sup> *Enterococcus* and 1,1x10<sup>5</sup> *Clostridium*; the sample 2 presented absence of *Enterococcus* and >2,4x10<sup>5</sup> *Clostridium*; and the sample 3, 1,0x10<sup>5</sup> *Enterococcus* and 2,4x10<sup>5</sup> *Clostridium*. *Salmonella* spp. was not detected in any sample analyzed. For sample 1, the BOD was 113,9 mg of oxygen/L, the COD of 6184,09 mg of oxygen/L, with turbidity of 24000 NTU, the pH 6,0 and apparent color of 100000 uH. For the sample 2, the BOD was 4334,59 mg of oxygen/L, the COD was not determined, with turbidity of 16800 NTU, the pH 6,38 and apparent color of 6000 uH. In sample 3, the BOD was 73,3 mg of oxygen/L, the COD was 2172,20 mg of oxygen/L, with turbidity of 4370 NTU, the pH 6,90 and apparent color of 16700 uH. The microbiological contamination was relevant, indicating high destructive potential for the environmental and healthy human population.

**FEMS-3132**

**Environmental microbiology - 2**

**MICROBIOLOGICAL AND PHYSICAL-CHEMICAL CHARACTERIZATION OF  
FLUSHING WATER FILTER FROM WATER TREATMENT PLANTS IN BRAZIL**

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Water treatment plants (WTP) yields waste as decanters sludge and flushing water filter (FWF). The objective of this study was characterizing FWF for presence of *Enterococcus* spp., *Clostridium* sulphite-reducing and *Salmonella* spp.. For physical-chemical parameters Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), turbidity, pH, apparent color, AWWA and WEF 2005 were used. The results showed in WTP-A (collection one), most probable number (MPN) of microorganism per 100mL was  $1,2 \times 10^2$  *Enterococcus* and  $>2,4 \times 10^3$  *Clostridium*; *Enterococcus* and  $1,1 \times 10^4$  *Clostridium*. BOD was 4,8 oxygen/L, COD 218,30mg oxygen/L, turbidity 754 NTU, pH 6,07 and apparent color 300 uH; In WTP-B, for collection 1, absence *Enterococcus*,  $7,0 \times 10^1$  *Clostridium*; BOD was 3,2 mg oxygen/L, COD 294,5 mg oxygen/L, turbidity 3990 NTU, pH 6,07 and apparent color 3000 uH. For collection 2, absence *Enterococcus* and  $4,3 \times 10^2$  *Clostridium*; BOD 2,43 mg oxygen/L, COD 1,3 mg oxygen/L, turbidity 881 NTU, pH 6,57 and apparent color 5500 uH; In WTP-B, for collection 2, absence *Enterococcus* and *Clostridium*; BOD 5,1 mg oxygen/L, COD 106,8 mg oxygen/L, turbidity 889 NTU, pH 6,78 and apparent color 4500 uH. For collection 3,  $3,9 \times 10^2$ ; BOD 11 mg oxygen/L, COD 628,83 mg oxygen/L, turbidity 1600 NTU, pH 6,98 and apparent color 54 uH;  $4,0 \times 10^1$  *Enterococcus* and  $4,0 \times 10^1$  *Clostridium*; BOD 3,7 mg oxygen/L, COD 170,9 mg oxygen/L, turbidity 190 NTU, pH 7,11 and apparent color 1100 uH. *Salmonella* spp. was not isolated. The microbiological contamination was relevant and high levels of physical-chemical parameters were detected in the flushing water filter, indicating destructive potential for the environment and population.

**FEMS-2770**

**Environmental microbiology - 2**

### **ENTERIC VIRUSES INACTIVATION BY GAMMA IRRADIATION**

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#### **Background**

Enteric viruses, like norovirus and adenovirus are frequently detected in environmental systems and have been implicated in many outbreaks. Normally, the viruses exist in the environment and acquire certain capability to endure conventional treatment processes. Alternative disinfection methods must be developed.

#### **Objectives**

The main goal of this study was to investigate the inactivation of murine norovirus type 1 (MNV-1), as a Norovirus surrogate, and human adenovirus type 5 (AdV-5) by gamma irradiation.

#### **Methods**

Six different substrates: Phosphate buffer saline (PBS), demineralized water, tap water, fetal bovine serum (FBS) and aqueous solutions of 10% and 50% FBS, were inoculated with MNV-1 and AdV-5 and irradiated in a Co-60 source at several doses (1 up to 10 kGy). The inactivation of viral particles was tested by plaque assay using Raw 264.7 and A549 cells. The D<sub>10</sub> values were estimated for each virus and substrate.

#### **Conclusions**

A reduction on MNV and AdV titers of 4 log<sub>10</sub> PFU/ml was achieved after irradiation at 3 kGy on PBS and water suspensions. However, MNV and AdV were approximately 3 times more resistant to gamma irradiation when irradiated in FBS, and at a dose of 10 kGy it was detected the presence of infective viral particles. The observed D<sub>10</sub> values ranged between 0.76 kGy (water) and 3.22 kGy (10% FBS) for MNV; and between 0.87 kGy (PBS) and 2.94 kGy (FBS) for AdV. These results show that inactivation of MNV-1 and AdV-5 by gamma radiation strongly depends on the substrate where the viruses are inoculated.





**RECOVERY OF AN ACETATE-CONSUMING SULFATE REDUCING  
CONSORTIUM AT ACIDIC CONDITIONS**

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**Background**

Sulfidogenic reactors have been used to eliminate dissolved metals from industrial wastewaters, due to their possibility to obtain metal sulfides. Sulfate reducing bacteria (SRB) are responsible for the production of sulfide which reacts with dissolved metals, forming precipitates (metal sulfides). However, the major drawback of sulfidogenic reactors is that acetate cannot be degraded completely, reducing the reactor's efficiency, another problem is that metal containing wastewaters are usually acidic (pH 2-4).

**Objectives**

The objective of this work was to obtain a consortium from a natural acidic environment (an abandoned sulfur mine) able to work at low pH (<4) and produce sulfide from the complete oxidation of the substrate.

**Methods**

The enrichments were preformed from sediments cultured in anaerobic bottles (120 mL) and fed with acetate, glycerol, or lactate, and sulfate. The pH was initially 3 or 4, and we evaluated their capability to completely consume the substrate and produce sulfide. When the enrichment consumed the substrate and produced sulfide, we took 10 or 20 % (v/v) as an inoculum to develop a new enrichment of sulfate-reducing consortium.

**Conclusions**

Supernatant (only the liquid part) used as inoculum (10-20%) was not favorable for the growth of SRB. The slurry (combination of sediment and liquid) used as inoculum (20%) promoted the sulfate-reducing activity at acidic pH. Acetate, used as the sole electron donor, was not suitable for the SRB consortium. Complex electron donor, such as glycerol or lactate, was more suitable to obtain a sulfate-reducing consortium at low pH able to consume acetate and produce sulfide.

**FEMS-1954**

**Environmental microbiology - 2**

**BACTERIAL DIVERSITY AND DYNAMICS FROM START-UP TO STEADY CONDITIONS IN A FULL-SCALE MUNICIPAL SOLID WASTE (MSW) ANAEROBIC REACTOR BY 454 PYROSEQUENCING TECHNOLOGY.**

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**Background**

Anaerobic digestion is an environmentally acceptable means of reducing and stabilizing organic fraction from MSW. Functioning and stability of an anaerobic reactor is directly related to the microbial community within it.

**Objectives**

The bacterial diversity of a full scale anaerobic reactor treating MSW from Madrid (Spain) was investigated using high-throughput 454 pyrosequencing technology.

**Methods**

The digested MSW samples were taken in two moments of the treatment: start-up phase and steady- state conditions. The bacterial 16S rRNA genes were amplified and sequenced using 454 pyrosequencing FLX machine.

**Conclusions**

118,392 bacterial 16S rDNA reads were yielded and after trimmed 35,981 (start-up phase) and 18,290 reads (steady conditions) were clustered into OTUs. Fifteen phyla were identified: *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, SR1, *Synergistetes*, *Planctomycetes*, *Tenericutes*, *Deinococcus-Thermus*, *Acidobacteria*, *Chlorobi*, *Cyanobacteria* and *Gemmatimonadetes*. Shifts in the bacterial communities were observed and proteolytic bacteria dominated during all the process, being the major diversity identified in the steady-state condition of the reactor. Fermentative bacteria belonged to *Bacteroidetes* and *Firmicutes* phyla predominated, and a great number of reads were identified to genus level. *Proteiniphylum*, *Gallicola* and *Fastidiosipila* genera carried out most biodegradation processes during the MSW treatment. A great number of genera are involved in the

sugars and carbohydrates metabolisms, although its coverage is low. The high concentration of ammonium appears to be the reason for the prevalence of the proteolytic bacteria. The use of the next generation sequencer technology revealed a great diversity of rare organisms and increased our knowledge about the dynamics of bacterial communities in anaerobic MSW reactors.

**FEMS-0884**

**Environmental microbiology - 2**

**BACTERIAL DIVERSITY IN RHIZOSPHERE AND SOIL ON MINING WASTE IN THE ARID ENVIRONMENT OF THE SAHARA**

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**Background**

Relatively few studies have been devoted to the microbial diversity of the Sahara. In this study, we have investigated an arid area located in south-west Algeria. In this area, spoil heaps of coal mine waste are colonized by a Saharo-Mediterranean vegetation dominated by *Caroxylon* sp.

**Objectives**

As part of an on-going study aiming to understand the role of micro-organisms in the adaptation of this plant to the site, we have studied its associated microflora.

**Methods**

The bacterial community was investigated by fingerprinting (RISA). The diversity was much higher in the *Caroxylon* rhizosphere than the surrounding soil, both on the waste heaps and in the desert soil. Libraries of 16S rRNA gene were obtained from rhizospheric and non rhizospheric soil-extracted DNA. Sequences from *Firmicutes* and *Actinobacteria* were abundant in all libraries. As expected from the fingerprints, the diversity was higher in the rhizosphere libraries, with 1.6 more phyla detected than in non rhizospheric soil.

**Conclusions**

Depending on the library, 25 to 65 % of the sequences had percentages of identity with known species below the 97% threshold. Potential new species are located in very diverse phyla. The most distant ones (less than 90% identity) belong to *Acidobacteria*, *Chloroflexi*, *Deltaproteobacteria*, *Cyanobacteria* and *Nitrospirae*. The results show that we have only begun to explore the diversity of soil bacteria in the arid environment of the Sahara.



**FEMS-0711**

**Environmental microbiology - 2**

**POTENTIAL OF CADMIUM RESISTANT AND BIPHENYL UTILIZING MARINE BACTERIA IN BIOREMEDIATION**

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**Background**

Utilization of aromatic compounds by marine microbial communities in nutrition and pollution stress has evolved several catabolic pathways for bioremediation. Of which, biphenyl and cadmium are highly toxic compounds having harmful effects on flora, fauna, and mankind.

**Objectives**

The main objective of this study was to find out the catabolic potential and pathway of marine bacteria in aerobic utilisation of biphenyl as sole carbon and energy source under toxic metal (cadmium) stress.

**Methods**

Two bacterial strains *Pseudomonas aeruginosa* (JP-11) and *Achromobacter xylosoxidans* (JP-22) were isolated from the coastal regions of Odisha, India, which could resist up to 1000 ppm and 630 ppm of cadmium respectively. They utilized 97.88 % and 89.6 % of biphenyl respectively. Combination of chromatographic and spectrophotometric techniques identified 2-Hydroxy-6-oxo-6-phenylhexa-2, 4-dienoate and benzopropanol as the intermediate product of biphenyl metabolism in JP-11 and JP-22 respectively. Biosurfactant production was observed which increased the bioavailability of this hydrophobic compound into the cells resulting in their effective removal from the environment. Of both the isolates, the partial structure of the biosurfactant produced by JP-11 was similar to that of rhamnolipid which showed an upregulation of rhamnolipid synthesis gene (*rhlAB*) with increased biphenyl concentration (upto 200 ppm).

**Conclusions**

Thus, these bacterial isolates can be efficiently utilized for bioremediation of the contaminated sites with toxic metal and organic pollutants.

FEMS-1596

Environmental microbiology - 2

## DIVERSITY OF OIL DEGRADING BACTERIA FROM GEOGRAPHICALLY DISTANT REGIONS

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### Background

An investigation of oil degrading bacteria is significant for practical and fundamental reasons. The diversity of metabolic systems allows these microorganisms to utilize a wide spectrum of substrates and produce different biological active substances.

### Objectives

The main aim of this research was to characterize oil degrading bacterial strains isolated from different geographical regions (Belarus, Libya, Iraq, and Antarctic).

### Methods

Physiological, biochemical and molecular genetic methods were used.

### Conclusions

Taxonomy of 22 oil degrading bacterial strains was established. They were determined as *Rhodococcus pyridinivorans*, *R. opacus*, *R. erythropolis*, *Bacillus licheniformis*, *B. beijingsensis*, *B. flexus*, *Arthrobacter* sp., *Micrococcus* sp., *Enterobacter* sp., *Planococcus maitriensis*, *Acinetobacter radioresistens*, *Pseudomonas* sp., *P. stutzeri*, *Dietzia* sp., *Deinococcus* sp..

*R. pyridinivorans* 5Ap was shown to have the widest spectrum of utilizing substrates (it utilized 19 investigated organic substrates). Different strains were able to grow under extreme conditions. For example, *B. licheniformis* FD9 grew at 54 °C; *P. stutzeri*, *R. erythropolis*, *Arthrobacter* sp. - at 4 °C; *Enterobacter* sp. FD1 - pH=4, *B. beijingsensis* FD4 - pH=11, *P. maitriensis* FD3 – with sodium chloride concentration up to 7 %; *A. radioresistens* and *Deinococcus* sp. were UV-resistant.

It was shown the presence of naphthalene catabolic plasmids in the strains of *R. pyridinivorans* and *R. opacus*.

The ability of oil degrading bacteria to produce the surfactants was shown. The most effective production of surfactants was determined for *R. erythropolis* A29-k1 when it was cultivated on mineral medium with hexadecane. Lichenisyn biosynthesis genes were found in *B. licheniformis* FD9.



**FEMS-1871**

**Environmental microbiology - 2**

## **MICROBIOLOGICAL INVESTIGATION OF MUD VOLCANOES FROM EASTERN ROMANIA FLUIDS**

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### **Background**

The largest areas of terrestrial mud volcanoes in Europe are located in the Carpathian Mountains in eastern Romania. Till now the few microbiological investigations were focused on the study of Archean communities implicated in methane- and hydrocarbon cycling.

### **Objectives**

The purpose of this study was to isolate, identify and characterize the pathogenic and resistance features of bacterial strains isolated from the bubbling fluids expelled from the mud volcanoes from Buzau in order to establish the contribution of these protected environments to the natural reservoirs of resistance and pathogenicity.

### **Methods**

All water samples (500 ml) were collected according to current ISO guidelines using sterile glass bottles, at a depth of approximately 30 cm and stored in cold bags at 4°C until analysis. The samples were diluted and analysed through the standardized membrane filtration method. The identification of aerobic strains obtained in pure culture was based on Gram staining, oxidase reaction and API galleries.

Simultaneously, the samples were processed for DNA extraction and molecular assays based on ribosomal DNA amplifications with general bacterial 16S rRNA primers. The isolated strains have been investigated for antibiotic susceptibility and for the production of cell-associated (adherence to inert and cellular substratum, biofilm development) and soluble, enzymatic (hemolysins, lecithinase, lipase, caseinase, gelatinase, amylase, esculin hydrolysis, DN-ase) virulence factors, using phenotypic (disk diffusion method, selective media for enzymatic factors production) and PCR-based methods.

## **Conclusions**

The virulence and resistance profiles of cultivable strains were very poor, suggesting the absence of any selective pressure agents in the investigated areas.

**FEMS-1097**

**Environmental microbiology - 2**

## **IDENTIFICATION AND CHARACTERIZATION OF GENES INVOLVED IN SIDEROPHORE PRODUCTION OF PANTOEA ANANATIS, THE CAUSATIVE BACTERIUM OF RICE SHEATH ROT**

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### **Background**

*Pantoea ananatis* is known to cause disease in maize, eucalyptus, onions, and rice. This bacterium is also known as an opportunistic human pathogen. *P. ananatis* PA13 is a rice grain and sheath rot bacterium, which produces an antibacterial compound.

### **Objectives**

In the present study, we have tried to identify and characterize genes involved in antibacterial activity in *P. ananatis* PA13.

### **Methods**

We performed a large-scale, *mariner* transposon-based genetic screening and gene replacement strategy based on homologous recombination. Antibacterial activity was monitored against *P. ananatis* HY02, onion center rot bacterium, and *Yersinia enterocolitica*.

### **Conclusions**

A number of proteins that involved in transcription, catalytic enzymes, siderophore transporting systems, and TonB-dependent transporter system were found to affect the antibacterial activity of strain PA13. These data indicate the iron uptake is a major factor of antibacterial activity in *P. ananatis* PA13. We focused then siderophore biosynthetic genes. *P. ananatis* has genes homologous to siderophore biosynthesis, aerobactin (*iucABCD*), alcaligin (*alcA*) and rhizobactin (*rhbF*). Single mutant of  $\Delta iucABC$  exhibited slight reduction of antibacterial activity, whereas  $\Delta alcA$  and  $\Delta rhbF$  showed normal antibacterial activity. This result led to generation of double and triple mutants of the genes. Mutants  $\Delta iucABC\Delta rhbF$  and  $\Delta iucABC\Delta alcA$  exhibited strong reduction in antibacterial activity, whereas mutant  $\Delta alcA\Delta rhbF$  showed normal antibacterial activity. These data indicate that siderophores play an important role in antibacterial activity of *P. ananatis* PA13. This work will give novel insights into iron

uptake regulation and contribute to a better understanding of how these genes function to regulate iron uptakes.

**FEMS-2504**

**Environmental microbiology - 2**

## **HYDROGEN PRODUCTION AND VFA ACCUMULATION IN A CONTINUOUS STIRRED TANK REACTOR USING MOLASSES WASTEWATER AS FEEDSTOCK**

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### **Background**

Molasses wastewater as a sugar-rich waste is a liquid by-product generated in large amount from food industries. Molasses wastewater is one of promising feedstock for bioenergy production because it has high strength of organics and relatively high bioavailability.

### **Objectives**

For bioenergy production and wastewater treatment simultaneously, two-stage fermentation processes employing hydrogenesis and methanogenesis have been developed. Hydrogenesis has advantage of volatile fatty acids (VFAs) as main byproducts as well as hydrogen, and VFA production is necessary to stabilize the waste stream in the secondary stage for methane production. Hydrogen production and VFA production was characterized in a continuous stirred tank reactor (CSTR) fed molasses wastewater as feedstock.

### **Methods**

The CSTR reactor achieved a stable performance at an organic loading rate from 20.3 to 36.1 g-COD·L<sup>-1</sup>·d<sup>-1</sup>. Average gas production rate was 1.2 L/d with a hydrogen concentration of 33%. The maximum hydrogen production rate was 1.02 L-H<sub>2</sub>·L<sup>-1</sup>·d<sup>-1</sup> at 31.0 g-COD·L<sup>-1</sup>·d<sup>-1</sup>. The abundant VFAs were butyric acid (50%) and acetic acid (38%). Total VFA production kept at around 7,135 mg/L level during operation period. Active microbial community was analyzed using rRNA-based massively parallel sequencing technique. Clostridiales, *Lactobacillus* and *Clostridium* were relatively abundant in the CSTR reactor. It has been reported that appearance of *Clostridium* species was in accordance with increase of butyrate and acetate concentration. *Lactobacillus* species has been known to be involved in anaerobic lactate degradation to acetate.

### **Conclusions**

VFAs produced in the CSTR reactor can be utilized as substrates for methane production in downstream processes.

**FEMS-2508**

**Environmental microbiology - 2**

**STARVATION EFFECT ON METHANE PRODUCTION IN AN UPFLOW ANAEROBIC SLUDGE BLANKET REACTOR**

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**Background**

Biohydrogen and biomethane can be produced in Upflow Anaerobic Sludge Blanket (UASB) reactors treating high strength wastewaters such as molasses wastewater and distillery wastewater.

**Objectives**

Even though many researches have been reported on the performance of UASB reactors and their microbial properties, there is little information about the effect of starvation on the UASB performance. In this study, the effect of starvation on methane production was characterized in an UASB reactor.

**Methods**

Anaerobic digested sludge was used as an inoculum source, and the effluent (10,000 mg-COD/L) from an hydrogen-producing UASB reactor treating molasses wastewater was used as influent wastewater for the methane-producing UASB reactor. The UASB reactor was operated for 18 days at hydraulic retention time of 3 d. After confirming the UASB performance reached at steady-state, the UASB was maintained at starvation condition for 14 d or 30 d. After starvation period, the UASB was re-operated by feeding the influent without re-inoculation, and COD removal and methane production were compared with their performance at steady-state (COD removal and methane production rate were 98% and  $0.91 \text{ L-CH}_4 \text{ L}^{-1} \text{ d}^{-1}$ , respectively).

**Conclusions**

After 3-days of 14 d-starvation, COD removal and methane production rate were 87% and  $1.51 \text{ L-CH}_4 \text{ L}^{-1} \text{ d}^{-1}$ , respectively. The UASB performance improved speedy, and the UASB performance could be recovered by 15-days operation after restarting. However just after 30 d of starvation, no COD removal and methane product was observed, and over 27-days operation was necessary to recovery the UASB performance. These findings can be used to plan the operation strategy of methane-producing UASB reactors.

**FEMS-0667**

**Environmental microbiology - 2**

## **APPLICATION OF BIOLOG PHENOTYPING MICROARRAYS FOR ASSESSING THE FUNCTIONAL DIVERSITY OF ENVIRONMENTAL STRAINS OF BACILLUS**

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### **Background**

The ability to link genotypes to corresponding phenotypes is of interest in biotechnological manipulation of metabolic pathways. In order to assess a rapid functional and phenotypic profiling under different metabolic conditions, a high-throughput approach has been developed by BIOLOG Inc. (Hayward, CA): the Phenotype Microarray (PM) technology.

### **Objectives**

The objective of the study was to determine phenotypic changes in environmental strains of *Bacillus* by Phenotyping MicroArrays.

### **Methods**

The four *Bacillus* strains were tested on PM 96-well plates (PM01-02 carbon sources, PM03 nitrogen sources, PM04 phosphorus and sulfur sources, PM09 with osmolytes, PM10 with another pH, PM11-13 with various antibiotics). *Bacillus subtilis* ATCC as reference strain was used to determine the differences between strains. Data were analyzed with Omnilog-PM software. The software suite called 'DuctApe' which analyzes and links together both the genomic and the phenomic data and suggests genetic explanations of metabolic phenotypes was also used.

### **Conclusions**

The environmental isolates as compared to the reference strain were found to be the most metabolically versatile strains, either by looking at the number of 'more active' metabolic features or by the proportion of active compounds for each category. The strains showed higher proportion of active compounds for carbon and nitrogen sources and in the resistance to chemical agents. The DuctApe software is good tool for the visualization of PM data, the exploration of both the genomic information and the phenotypic expression of *Bacillus* spp. tested. Phenotypic profiling is an essential step for understanding genotype differences for environmental microorganisms of interest in contaminant remediation, biofuels production, and climate change.

**FEMS-2975**

**Environmental microbiology - 2**

**THE STRUCTURAL AND FUNCTIONAL BIODIVERSITY OF MICROORGANISMS  
IN SOIL UNDER EX SITU AIDED PHYTOSTABILIZATION**

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**Background**

The high toxicity of heavy metals causes the need to remove them from the contaminated soil using minimally invasive remediation solutions. One of them seems to be aided phytostabilisation.

**Objectives**

The aim of this project was to investigate the impact of organic by-products as heavy metals stabilizers in soil on the biodiversity of microorganisms under *ex situ* aided phytostabilisation.

**Methods**

Green waste compost and pulp from the processing of grain were introduced into highly contaminated with Zn, Pb and Cd soil. As a phytostabilizer grass *Festuca arundinacea* was used. After 18 weeks the activity of enzymes: dehydrogenases, phosphatases and urease as well as the structural and functional biodiversity of microorganisms were tested. The biomass of grass after crop was also analysed.

**Conclusions**

The activity of enzymes in soil supplemented with grain was significantly higher in comparison with their activity in soil amended with compost. Simultaneously, the addition of grain into soil caused the highest increase the metabolic activity and biodiversity of microorganisms in comparison with soil amended with green compost. Additionally, after plant cutting the grass achieved the highest biomass in soil with grain.



**FEMS-0826**

**Environmental microbiology - 2**

## **REMOVAL OF HEXAVALENT CHROMIUM BY OCHROBACTRUM SP. CUST210-1 AND CHROMATE REDUCTASE**

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### **Background**

Chromium is extensively used in resistant alloys, electroplating, dye productions and leather tanneries. Hexavalent chromium (Cr<sup>6+</sup>) is a toxic, mutagenic and carcinogenic chemical. Physicochemical processes have been applied in Cr<sup>6+</sup> treatment, however, their setup and operating costs are high for large-scale treatment. Based on this consideration, biotechnology is a potential alternative.

### **Objectives**

In this study, we would like to reveal the characteristics of *Ochrobactrum* sp. CUST210-1 and its crude chromate reductase in removing Cr(VI).

### **Methods**

We isolated chromium-resistant bacterium from leather industry wastewater by chemostat. Its chromate reductase was purified and further identified by SDS-PAGE. Characteristics of bacterial cell and chromate reductase for Cr(VI) removal were evaluated.

### **Conclusions**

A facultative *Ochrobactrum* sp. CUST210-1 was isolated and it achieved high removal efficiency at relatively low Cr(VI) concentration (<350 mg/L) under the aerobic condition. Cu<sup>2+</sup>, Zn<sup>2+</sup>, Ni<sup>2+</sup>, SO<sub>4</sub><sup>2-</sup> and Cl<sup>-</sup> with 10-150 mg/L did not affect Cr(VI) removal by CUST210-1. Thus, the cell technique could be applied in treating chromium-containing wastewater (e.g. leather industry wastewater). By SDS-PAGE analysis, we confirmed that crude chromate reductase was successfully isolated. NADH rather than carbohydrate was optimal e<sup>-</sup> donors for the activity of chromate reductase. Thus, immobilized enzyme technique could be applied in treating chromium-containing wastewater with low concentrations of organic compounds (e.g. electroplating wastewater). To our knowledge, this is the first report to demonstrate the application range by bacterial cell or immobilized enzyme in removing Cr(VI).

**FEMS-1693**

**Environmental microbiology - 2**

**INVESTIGATION OF METABOLICALLY ACTIVE BACTERIA LEVELS AROUND  
GÖKÇEADA ISLAND (THE NORTHERN PART OF AEGEAN SEA), TURKEY**

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**Background**

Determination of the metabolically active bacteria level in the studied area is essential in terms of describe of bacteria that contribute to the ecological in aquatic ecosystems. Metabolically active bacteria have a well-developed polysaccharide capsule whereas inactive bacteria rapidly release the capsule (the term "capsulated bacteria was used in reference to "capsule bearing bacteria").

**Objectives**

The aim of this study was to investigate total and metabolically active bacteria levels at the surface sea water samples taken the coastal and off shore areas which have chosen around Gökçeada Island, northern part of Aegean Sea (Fig.1).

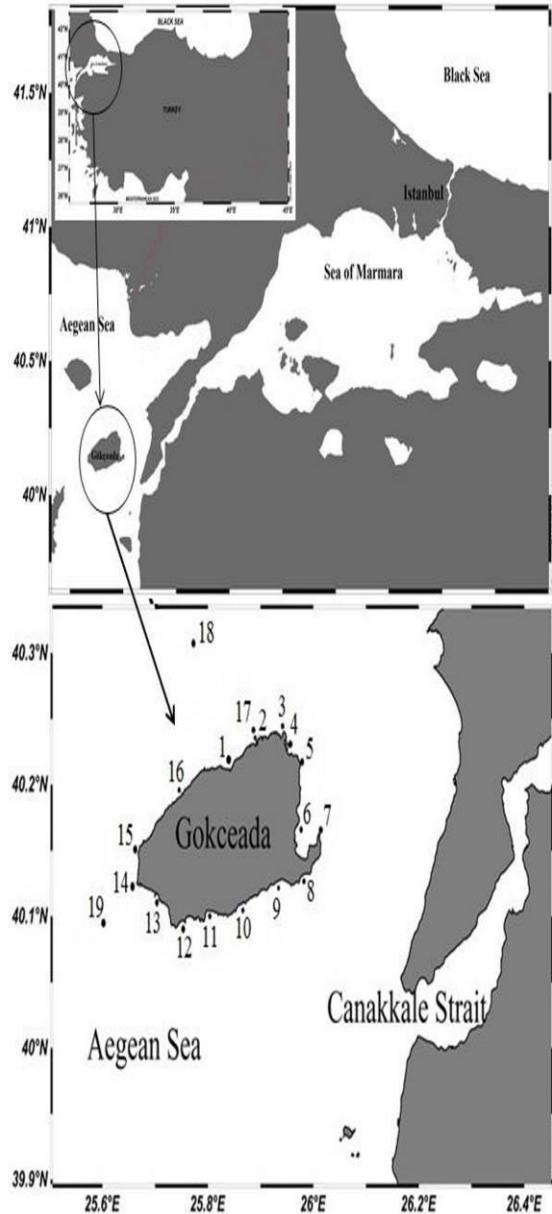


Fig. 1. Sampling area

## Methods

Sea water samples were taken 19 stations around Gökçeada Island (northern part of Aegean Sea) seasonally for the autumn, winter, spring as monthly for the summer in 12 times in total between March 2012 - November 2013. The frequency of the metabolically active bacteria was determined with modified staining technique comparing the number of the intact cells to the total (live and dead bacterial cells) number of bacteria (Plante and Shriwer, 1998; Stodereger and Herndl., 2001).

## **Conclusions**

The highest metabolically active bacteria percentage was determined to be 33.12% in the seawater samples which were taken from Aydıncık Bay in 2012 summer and the metabolically active bacteria levels in coastal station was found higher than the off-shore stations.

This work was supported by Research Fund of the Istanbul University as a PhD thesis project (Project No: 17653).

## **References**

- Plante, C.J., Shriwer, A.G., 1998, Journal of Experimental Marine Biology and Ecology, 229: 35-52.
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**FEMS-0544**

**Environmental microbiology - 2**

## **DEGRADATION OF NAPROXEN BY A SELECTED FUNGAL STRAIN**

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### **Background**

Pharmaceuticals are widely used for treating human and animal diseases. These active compounds and their metabolites can enter aquatic environments via urine, feces, discharge from production facilities and hospital wastes (Kosjek et al. 2005). Naproxen and its sodium salt are members of the  $\alpha$ -arylpropionic acid group of non-steroidal anti-inflammatory drugs. Due to excessive usage of naproxen, this drug has been detected in aquatic environments and even in drinking water (Marco-Urrea et al. 2010).

### **Objectives**

The principal aim of this research was assessment of naproxen degradation capabilities of four fungal strains, include two white-rot fungi (*Phanerochaete chrysosporium*, *Funalia trogii*), yeast *Yarrowia lipolytica*, filamentous fungus *Aspergillus niger* and identification of byproducts.

### **Methods**

The LC/MS spectra were taken on a Waters Micromass ZQ connected with Waters Alliance HPLC, using ESI (-) method, with C-18 column. For the structural elucidation of isolated metabolites, low resolution LC-MS and NMR spectroscopy have been used. <sup>1</sup>H (400 MHz) was recorded employing a VARIAN MERCURY 400 MHz FT spectrometer.

### **Conclusions**

*Aspergillus niger* found to the most efficient strain with %98 degradation rate. Two main byproducts of degradation, O-desmethylnaproxen and 7-hydroxynaproxen were identify using LC/MS and <sup>1</sup>H-NMR.

### **References**

Kosjek T, Heath E, Krbavcic A (2005) Determination of non-steroidal anti-inflammatory drug (NSAIDs) residues in water samples. Environ Int 31:679–85.

Marco-Urrea E, Pérez-Trujillo M, Blázquez P, et al. (2010) Biodegradation of the analgesic naproxen by *Trametes versicolor* and identification of intermediates using HPLC-DAD-MS and NMR. *Bioresour Technol* 101:2159–66.

## THE INFLUENCE OF ENDOPHYTIC BACTERIA IN WHEAT PLANTS FERTILIZED WITH NITROGEN

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### Background

The plant growth-promotion bacteria improve nutrient uptake, reduce fertilization cost and also minimize the environmental pollution by decreasing nitrogen leaching. However, there is not enough data about plant growth-promotion bacteria and grasses interaction, especially with endophyte bacteria.

### Objectives

The aim of this work was evaluate the possible benefits of three diazotrophic endophytic bacteria about the role in the nitrogen metabolism, ability to synthesize plant hormones and the influence of different N level on wheat plant.

### Methods

Wheat plants were fertilized with three nitrogen levels (no-fertilizer, half recommended N-fertilizer and full recommended N-fertilizer) and treated with different *nif* H<sup>+</sup>, endophytic bacteria: IAC-AT-8 (*Azospirillum brasilense*), IAC-HT-11 (*Achromobacter insolitus*) and IAC-HT-12 (*Zoogloea ramigera*). The enzymes nitrate reductase, glutamine synthetase and chlorophyll content analyses were analyzed as well as the IAA production by the strains. Before the harvest the nitrogen and the growth promotion were also evaluated.

### Conclusions

All the three bacteria were able to modify the nitrate reductase and the glutamine synthetase activities and improve the chlorophyll content, and promote the root and shoot weight. The strains also produced IAA in pure cells culture. The N content and the N-use efficiency index were dependent on the endophytic bacteria strain and on the plant tissue assessed. In general, the performance of the above parameters was strongly affected by the nitrogen rate. The different responses suggest that the successful colonization and the growth promotion are achieved by distinct mechanisms.





**FEMS-1945**

**Environmental microbiology - 2**

## **ANTIBIOTICS PROMOTE AGGREGATION WITHIN AQUATIC BACTERIAL COMMUNITIES**

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### **Background**

The release of antibiotics (AB) into the environment poses several threats for human. Knowledge on the impact of AB on natural bacterial communities is missing both in terms of spread and evolution of resistance mechanisms, and of modifications of natural community composition and productivity.

### **Objectives**

We performed a chemostat-based experiment with 4 coexisting bacterial strains mimicking a freshwater bacterial community to study their response to antibiotics in low and high doses.

### **Methods**

Bacterial abundance rapidly decreased by 75% in the presence of AB, independently of their concentration, and remained constant until the end of the experiment. The bacterial community was mainly dominated by *Aeromonas hydrophila* and *Brevundimonas intermedia* while the other two strains, *Micrococcus luteus* and *Rhodococcus* sp. never exceed 10%. Interestingly, the bacterial strains, which were isolated at the end of the experiment, were not AB-resistant; in addition reassembled communities composed of the 4 strains, isolated from treatments under AB stress, significantly raised their performance (growth rate, abundance) in the presence of AB compared to the communities reassembled with strains isolated from the treatment without AB. By investigating the phenotypic adaptations of the communities subjected to the different treatments, we found that the presence of AB significantly increased co-aggregation by 5-6 fold.

### **Conclusions**

These results represent the first observation of co-aggregation as a successful strategy of AB resistance based on phenotype in aquatic bacterial communities, and can represent a fundamental step in the understanding of the effects of AB in aquatic ecosystems.

**FEMS-1950**

**Environmental microbiology - 2**

**ISOLATION AND CHARACTERIZATION OF THERMOTOLERANT PETROLEUM-  
OXIDIZING BACTERIA FROM GEOGRAPHICALLY REMOTE REGIONS**

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**Background**

Bioremediation of oil-polluted ecosystems in the hot climate regions can be performed by microbial consortia of thermotolerant bacteria.

**Objectives**

The objective of our work was to isolate thermotolerant bacteria from soil and water samples, physiological and biochemical analysis and taxonomic characterization of the bacteria.

**Methods**

In the work we used such methods as bacterial cultivation, DNA isolation, PCR, electrophoresis in agarose gel, direct sequencing, phylogenetic analysis, IR-spectroscopy.

**Conclusions**

Among 85 petroleum-oxidizing bacteria isolated from soil and water samples of Russia, Kazakhstan and Antarctica 15 thermotolerant gram-positive strains have been found which can utilize petroleum and particular hydrocarbons at 45°C and higher. The isolates were identified as the members of genera *Gordonia*, *Paenibacillus* and *Rhodococcus* and were closely related to known species of those genera. The *alkB* gene (encodes for alkane 1-monooxygenase) has been determined in all *Gordonia* isolates. Most thermotolerant isolates of genera *Gordonia*, *Paenibacillus* and *Rhodococcus* utilize petroleum in salt-enriched media.

The strains *Rhodococcus* sp. 5Ap, *Rhodococcus* sp. Par7 and *Gordonia* sp. 1D were the most effective thermotolerant petroleum-oxidizing bacteria at 45°C – 27%, 14% and 20% of petroleum, respectively. At the temperature 24°C the most effective petroleum-oxidizing strain was *Gordonia* sp. 1D – up to 59% of petroleum.



**ADAPTATION OF ARTHROSPIRA SP. PCC 8005 TO NITROGEN STARVATION:  
UNEXPECTED EXPRESSION OF HETEROCYST-LIKE PROTEINS**

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**Background**

Among the most spread microorganisms on Earth, cyanobacteria play a dominant role in nitrogen and carbon cycles. The main limiting factor in cyanobacterial growth results from essential nutrients deprivation (e.g., nitrogen starvation). To deal with that, cyanobacteria develop survival strategies, including morphological and metabolic changes.

**Objectives**

This study aims to investigate the morphological and metabolic changes within *Arthrospira* sp. PCC 8005 under N starvation.

**Methods**

In order to characterize the biological response of this strain, a multidisciplinary investigation was carried out (*i.e.*, proteomic, electronic microscopy, intracellular compounds assay,...).

**Conclusions**

*Arthrospira* sp. PCC 8005 underwent to metabolic reprogrammings of C and N metabolisms. Proteomic investigations suggested a degradation of the phycobilisomes, cyanophycine and potential endogenous N sources (e.g., nitrile, urea, cyanates & formamide) to counteract the lack of N in the medium. As N metabolism is regulated thanks to N-to-C ratio, N starvation results in C over-excess, which was stored as glycogen as indicated by proteomic and transmission electronic microscopy investigations. TEM confirmed proteomic results as shown in the decreased of thylakoids, cyanophycin, and in glycogen granules accumulation. *Arthrospira* sp. PCC 8005 exhibited a survival capacity during long-N starvation, likely by maintaining a basic activity in cells named HFN<sup>-</sup>, probably to sustain colonies viability. Interestingly, proteomic results showed an increased of heterocyst-like proteins (HetR & HglK), while the colonies are non-heterocystous. In order to characterize the role of these heterocyst-like proteins during N starvation in our strain, we carried out immunolocalization of HetR, and studied its potential protein partners by co-immunoprecipitation.

**FEMS-2115**

**Environmental microbiology - 2**

**ANALYSIS OF EXTRACYTOPLASMIC FUNCTION SIGMA FACTORS IN THE  
PLANT GROWTH-PROMOTING RHIZOBACTERIA BURKHOLDERIA  
PHYTOFIRMANS PSJN**

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**Background**

Nutrient availability makes plant internal tissues and rhizosphere attractive spaces for microbial colonization. However, bacteria interacting with plants need to adapt to constant environmental changes, reacting to fluctuations in their immediate surroundings through global gene regulation. Sigma factors are dissociable subunits of RNA polymerase that regulate gene transcription initiation by recognition of specific promoter sequences. Their total number in the genome of a specific bacterial strain is usually determined both by its lifestyle and its genomic size. The extracytoplasmic function sigma factors (ECFsf) are the largest and more diverse group of these factors (classified into 43 subgroups), but little is known about their functions, although they are predominant in environmental and plant associated bacteria, which makes them interesting candidates for global gene regulation during plant colonization.

**Objectives**

The aim of this study was to inactivate and to characterize ECFsf present in the plant growth-promoting rhizobacteria *Burkholderia phytofirmans* PsJN.

**Methods**

This strain contains 18 ECFsf, which were inactivated by insertional mutagenesis.

**Conclusions**

Results suggested that at least one ECFsf could be related with growth and general metabolic processes in strain PsJN, meanwhile others ECFsf may be specifically involved in oxidative stress tolerance and biofilm formation, processes related to plant colonization by bacteria. In general, the results support a possible role of ECFsf in plant bacteria interactions at the level of the rhizosphere.

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**FEMS-0806**

**Environmental microbiology - 2**

## **MELANIN SYNTHESIS BY BACILLUS WEIHENSTEPHANENSIS STRAINS ISOLATED FROM SOIL IN NORTHEASTERN POLAND**

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### **Background**

Melanin is known for protecting living organisms from harmful physical and chemical factors, however, its synthesis is rarely observed among *Bacillus cereus sensu lato*. So far, only two melanin-positive wild strains, *B. thuringiensis* subsp. *dendrolimus* [1] and *B. thuringiensis* subsp. *kurstaki* [2], have been described, but melanin production by *B. weihenstephanensis* has not been reported yet.

### **Objectives**

*B. weihenstephanensis* isolates originated from soil in Northeastern Poland were investigated in order to assess physicochemical properties of a blackish-brown pigment they produced and the mechanism of its synthesis in relation to their genotypic and phenotypic characteristics.

### **Methods**

Phenotypic and genotypic investigation of the melanin producers were done using API system, Multi-locus Sequence Typing, 16S rRNA sequencing, and Pulsed-Field Gel Electrophoresis. Fourier transform infrared spectroscopy we used to compare produced pigment with commercial melanin. The mechanism of the pigment production was assessed by biochemical tests and the whole genome sequencing of one *B. weihenstephanensis* strain.

### **Conclusions**

For the first time, we report that psychrotolerant *B. weihenstephanensis* can produce melanin pigment, which is probably associated with laccase activity. These environmental isolates are closely related to the *B. weihenstephanensis* DSMZ 11821 reference strains. The ability of melanin synthesis by soil *B. weihenstephanensis* strains seems to be a local adaptation to a specific niche.

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**FEMS-3042**

**Environmental microbiology - 2**

## **MICROBIOLOGICAL ANALYSIS OF THE SURFACE OF THE UNDERGROUND REINFORCED CONCRETE STRUCTURES**

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### **Background**

In public buildings and facilities, in areas with high humidity and specific climatic conditions microbiological corrosion is an important factor affecting the reliability and durability of structures made of metal, concrete and reinforced concrete. Therefore, the problem of potential protection of building materials and structures is very complex and actual.

### **Objectives**

Estimation of the degree of contamination of reinforced concrete structures of Almaty subway by corrosive dangerous microflora.

### **Methods**

Isolation of corrosive dangerous microorganisms was performed by seeding selected water samples and swabs of concrete materials on selective media.

### **Conclusions**

7 stations of Almaty subway were examined in spring 2014. 31 samples were selected, including 5 water samples and 26 swabs from different visually damaged surfaces of reinforced concrete structures. Studies have shown that pH of the water samples and scrapings were neutral or alkaline. Heterotrophic bacteria, filamentous fungi and denitrifying microorganisms were found in almost all samples. Their number was high enough. This is an alarming fact, because of their intensive development can contribute to a change in environmental conditions that are favorable for the development of thione and sulfate-reducing bacteria, which are corrosive dangerous microorganisms. Further, these group of microorganisms may also participate in the destruction of the concrete.

Thione and sulfate-reducing bacteria were detected in small amounts. However, their presence shows that they can grow intensively in creating the favorable conditions and thereby contribute to the development of corrosion processes in reinforced concrete structures.



**FEMS-1855**

**Environmental microbiology - 2**

## **THE DIVERSITY OF MICROBIAL COMMUNITIES AT ACIDIC MINE WATER OUTLETS IS DRIVEN BY STOCHASTIC PROCESSES**

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### **Background**

Explanation how the vast microbial diversity with multiple concurrent organisms may persist at a single site is still elusive for microbial ecology. One of plausible hypotheses is that a large number of spatially separated microcommunities exist within each complex habitat. Thus, each real assembly consists of a small and largely random selection of organisms from the total observed diversity.

### **Objectives**

One of plausible ways to test these hypotheses is to compare microbial communities from homogeneous microhabitats that exist in multiple physically separated copies at a single site. We selected ferruginous stalactites growing in large groups at acidic mine drainage outlets as a model of such microhabitats.

### **Methods**

Two groups of microbial stalactites growing at a single acidic mine drainage outlet and six stalactites from other sites were sampled. Prokaryotic 16S rDNA was amplified using universal primers and microbial diversity was characterized by tRFLP and amplicon pyrosequencing.

### **Conclusions**

Both methods showed that simple and homogeneous microbial communities inhabited all stalactites, but species-level OTU composition substantially differed even between adjacent stalactites. Although the sampling sites influenced composition of microbial communities, all OTUs including the most abundant ones unpredictably alternated in all samples. As a result, a large portion of communities clustered independently of locality or other variables by both phylogenetic beta diversity and OTU abundance metrics. Thus, each community probably contains a random selection from the group of plausible species. The environmental traits influence the probability of the presence of individual species but do not determine composition of simple microbial assemblies.



**FEMS-1476**

**Environmental microbiology - 2**

**THE MICROBIOME OF THE ESTERO SALADO MANGROVE: A 16S TAG APPROACH TO DETERMINE DIFFERENCES IN BACTERIAL COMMUNITY, ON SEDIMENTS CONTAMINATED BY HEAVY METALS.**

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**Background**

Mangrove ecosystems are coastal estuarine systems confined to the tropical and subtropical regions. The Estero Salado mangrove located in Guayaquil, Ecuador, has suffered constant disturbances during the past 20 years, due to industrial wastewater release. Previous work determined that this estuary is one of the most disrupted on earth due to its relationship with anthropogenic disturbance.

**Objectives**

To determine the concentration of heavy metals and nutrients in surface sediments of the Estero Salado in equatorial summer season (February).

To determine the influence of high concentrations of heavy metals in terms of composition of the bacterial microbiome.

**Methods**

A proper understanding of the spatial variations of microbial communities will provide clues about the underline mechanisms that structure microbial groups. In the present study, the analysis of concentration of heavy metals, nutrients and tag sequencing of 16S rRNA V4 was conducted in surface sediments in two mangroves areas in Guayaquil. The first one under the influence of heavy metals and other pollution free

**Conclusions**

Using the high throughput sequencing method, revealed a detailed picture of the spatial variations of the bacterial community structure in the superficial sediment on Estero Salado mangrove.

heavy metals directly influence on bacterial community due to in the contaminated area had a relatively lower alpha-diversity than the area without its presence.

We were able to identify bacterial groups significantly enriched in specific locations. This is the first approach in a mangrove area using tag sequencing and correlating with heavy metals levels.

**FEMS-1641**

**Environmental microbiology - 2**

**BEHAVIOUR OF SOME BACTERIA ISOLATED FROM CITRUS RHIZOSPHERE  
INFESTED BY TYLENCHULUS SEMIPENETRANS, IN TUNISIA**

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**Background**

The cape Bon area (Tunisia) is the world leading producer and exporter of Maltese half blood oranges. However the sour orange (*Citrus aurantium*) rootstock is naturally infested by a phytopathogen nematode, *Tylenchulus semipenetrans*, a major nematode pest. Some bacteria and nematophagous fungi demonstrated a nematicidal effect.

**Objectives**

Only few studies have been conducted on the interaction between bacteria and nematodes in the rhizosphere of citrus trees. The aim of this study is to better understand the role of some bacteria in the complex microbial ecosystem of the Tunisian Citrus rhizosphere.

**Methods**

Sixty bacterial strains were isolated from Citrus root trees and egg masses of nematodes. The isolates were screened for i) their nematicidal activity on eggs and on second stage juvenile of *Tylenchulus semipenetrans*, ii) their antifungal activity and iii) their effect on the growth of sour orange roots. To identify the isolates rDNA and/or rpoB gene sequencing was carried out.

**Conclusions**

Among the 60 bacterial isolates, the majority belonged to either *Bacillus subtilis* or *Bacillus cereus*. However, the strains showed a great diversity in their effects i) on the plant host Citrus, ii) on the nematode *Tylenchulus semipenetrans*, iii) on the rhizosphere-associated fungi (i.e. the phytopathogen *Fusarium solani* and predatory nematodes *Monacrosporium cianopagum* and *Arthrobotrys conoides*). Almost 30%

showed antifungal activities against *F. solani*.

These bacterial strains could be used to improve the quality of soil when used in the field by mixing various bacteria, thereby combining antifungal activity and plant growth-promoting bacteria. Large-scale trials should be performed to confirm these promising results.

**FEMS-1653**

**Environmental microbiology - 2**

## **ICE NUCLEATION ACTIVITY IN THE WIDESPREAD SOIL FUNGUS**

### **MORTIERELLA ALPINA**

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### **Background**

Biological residues in soil dust are a potentially strong source of atmospheric ice nucleators (IN). So far, however, the sources and characteristics of biological - in particular, fungal - IN in soil dust have not been characterized.

### **Objectives**

Thus, the objective of this study is a regional investigation of the identity and relative abundances of culturable INA fungi in topsoils, an essential base for improving our understanding of the effects of microorganisms on climate and the hydrological cycle.

### **Methods**

Soil fungi, from a range of different land use and ecosystem types in south-east Wyoming, were analyzed for ice nucleation activity. Ice nucleation active (INA, i.e., inducing ice formation in the probed range of temperature and concentration) fungi were found to be both widespread and abundant, particularly in soils with recent inputs of decomposable organic matter. Across all investigated soils, 8% of fungal isolates were INA. All INA isolates initiated freezing at -5°C to -6°C, and belonged to a single zygomycotic species, *Mortierella alpina* (*Mortierellales*, *Mortierellomycotina*). The IN produced by *M. alpina* seem to be proteinaceous, <300 kDa in size, and can be easily washed off the mycelium.

### **Conclusions**

Ice nucleating fungal mycelium will ramify topsoils and probably also release cell-free IN into it. If these IN survive decomposition or are adsorbed onto mineral surfaces, these small cell-free IN might contribute to the as yet uncharacterized pool of atmospheric IN released by soils as dusts.

**FEMS-0679**

**Environmental microbiology - 2**

## **DEVELOPMENT OF GREEN ALTERNATIVE COATINGS TO PROTECT THE METALLIC STRUCTURES AGAINST THE MICROBIAL INDUCED CORROSION PROCESS**

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### **Background**

Microbial Influenced Corrosion (MIC) is a very aggressive form of corrosion with many proposed mechanisms for its prevention, but existing antifouling solutions include not environmentally friendly biocides. This fact has caused an urgent demand for greener, non-toxic or low-toxicity and longer lasting antifouling compounds and technologies.

### **Objectives**

BIOCORIN project, within FP7-ENVIRONMENT program, aims to develop a green alternative to the coatings and solutions used up to date for MIC corrosion protection and prevention in infrastructures.

### **Methods**

The project is based on a biological phenomenon commonly occurring in nature for microbial population regulation. Microorganisms secrete compounds that inhibit the growth of other microorganisms due to existing antagonism between microbial populations. First step was the isolation and identification of those microorganisms that play a major role in fouling and MIC for different environmental conditions. Several samples were collected from different climate locations for the isolation and identification of microbial populations from metal surfaces. As a result, several microorganisms belonging to bacteria, fungi and yeasts were isolated from the four

different samples and were identified by means of 16S and 18S rRNA gene sequencing.

#### Conclusions

The search of microorganisms with anti-MIC properties resulted in the identification of two candidates with the ability to synthesize compounds with antifouling properties. These candidates were integrated in a coating based on sol–gel technology to prevent the MIC corrosion phenomena in a green way. Currently, this technology is under evaluation by means of three case studies with different environmental conditions.



**FEMS-0680**

**Environmental microbiology - 2**

**METABOLOME ANALYSIS OF THE ANTIMICROBIAL COMPOUNDS SECRETED BY ENVIRONMENTALLY ISOLATED ANTI-MIC MICROORGANISMS**

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**Background**

Corrosion is a worldwide problem and the direct economic loss due to metal corrosion of infrastructures and equipments is estimated to exceed €1.32 trillion per year, which accounts for 3 to 4% of the Gross Domestic Product of industrialized countries.

Among corrosion types, Microbial Induced Corrosion (MIC) is involved in at least 10% of the corrosion of structures, and up to 50% in the case of subterranean pipes.

**Objectives**

BIOCORIN (New Biocoating for Corrosion Inhibition in Metal Surfaces) is a project funded by the European Union under the activities of the Seventh Framework Programme (FP 7-ENVIRONMENT) that aims to develop a green alternative to the coatings and solutions used up to date for MIC corrosion control. Some of the results of this project are presented here, with the identification of several relevant antifouling compounds secreted by environmentally isolated anti-MIC strains via a metabolomic approach.

**Methods**

Metabolites were extracted from three anti-MIC strains. 100 ml cultures incubated in Terrific Broth (TB) at 30°C for 24 h in the presence and absence of a pool of MIC extracts (inducers) originated from several climatic locations around Europe. Each culture broth was recovered and analyzed by means of LC-MS and GC-MS (Acquity UPLC - SYNAPT G2 HDMS system QToF with ESI injection and a Thermo Scientific Focus GC - DSQ II respectively).

**Conclusions**

The metabolomics analysis pointed out the differential expression of 2 compounds by GC-MS and more than 10 compounds for LC-MS (positive mode) between the analyzed anti-MIC strains.

**FEMS-2199**

**Environmental microbiology - 2**

**DETERMINATION OF FUNGUS PRESENT IN THE AIR TAKEN IN PM10 FILTERS  
TWO NETWORK STATIONS MONITORING AIR QUALITY IN THE VALLE DE  
ABURRÁ (MEDELLÍN – COLOMBIA)**

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**Background**

Air quality is an issue that has come to be of great concern in recent years due to increased production of particulate material. The presence of fungi in the air is partly responsible for some allergies, infections and diseases. Because of this there is a need to study fungal species associated with inhalable particulate matter and its effects on health.

**Objectives**

The aim of the research is to isolate the fungi present in PM10 filters in sampling two Network Monitoring Air Quality in Aburrá Valley, which presented the highest levels of pollution in the city. After isolation fungi are identified and characterized by the types of pathogens.

**Methods**

The microorganisms are absorbed into quartz filters that capture particles diameters smaller than 10 microns (PM10). The filters are exposed to atmospheric air for 24 hours in the HI-VOL PM10 equipment. Subsequently, the filter was incubated in enrichment medium, serial dilutions were made and plated on selective media for fungi (PDA and Sabouraud in this case). After growth, morphological identification is performed.

**Conclusions**

The results of the tests reveal the presence of *Cladosporium* sp., *Fusarium* sp., *Alternaria* sp. y *Rhodotorula* sp. These microorganisms are considered allergens and are associated with the generation of respiratory diseases like asthma, rhinitis, tracheitis, bronchitis, pneumonia, bronchopulmonary mycosis, among others. These findings demonstrate the importance of knowing the microorganisms associated with particulate material breathe as increased air pollutants in the atmosphere increases morbidity and mortality.

## **FEMS-1063**

### **Environmental microbiology - 2**

#### **DIFFUSION SANDWICH SYSTEM, A TOOL TO ACCESS THE MICROBIAL DARK MATTER**

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#### **Background**

Prokaryotes are the most abundant and diverse life form on Earth. However, to date, we know that only between 0.001% and 1% of total environmental bacteria have been cultivated through standard culture techniques and media, remaining then a rich diversity of bacterial taxa to be isolated and cultured, the so-called microbial dark matter.

#### **Objectives**

The aim of this study was to carry out a proof of concept of a system of miniature diffusion chambers which we have named Diffusion Sandwich System (DSS), as a cheap and user-friendly tool to gain access to previously uncultured bacterial taxa.

#### **Methods**

We did a comparative study of the cultivability and bacterial diversity which could be isolated by using the DSS and with the classical approach based on picking up bacterial colonies from Petri dishes. Further both culture-dependent approaches were compared with the bacterial diversity present in the same ecological niche but analyzed in-depth by pyrosequencing the metagenomic 16S rRNA gene.

#### **Conclusions**

The culturability obtained by using the miniaturized chambers was 2-log<sub>10</sub> units higher than with the classical approach. Unlike the classical approach, the relative abundances of taxa isolated with the DSS was in line with those actually present in the ecological niche, allowing in fact to isolate representatives of several underrepresented phyla such as *Acidobacteria*, *Verrucomicrobia* and *Gemmatimonadetes*, which constitute new taxa into these phyla.

**FEMS-2164**

**Environmental microbiology - 2**

## **MARINE PHAGES AS MARKERS OF REACTIVE TRANSPORT OF THE COLLOIDAL PARTICLES IN THE CRITICAL ZONE**

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### **Background**

Little is known about the factors determining the eligibility of marine phages as specific markers of hydrological flow and reactive transport of colloidal particles in the Earth's Critical Zone (CZ). Marine phages and their bacterial hosts are naturally absent in soil, can be applied as tracers at high concentration and be detected as little as one phage/mL in water.

### **Objectives**

Within the DFG Collaborative Research Center – AquaDiva we evaluated the effects of phage characteristics (morphology, surface property), water flow regimes as well as the role of mycelial networks on transport of marine phages in laboratory experiments.

### **Methods**

Phages were characterized by electron microscopy, dynamic light scattering and water contact angle analysis (CA) for their size/morphology, surface charge ( $\zeta$ ) and hydrophobicity. Sand-filled percolation columns and a modified high-throughput plaque assay combined with fluorescence microscopy counting were used to quantify phage deposition during percolation.

### **Conclusions**

Our data show that marine phages exhibit differences in their deposition efficiency depending on their size, surface charge and hydrophobicity. For instance, the bigger (60 nm), more hydrophobic (CA = 78°) and poorly charged ( $\zeta_{\text{TM, pH=7-8}} = -13 \text{ mV}$ ) PSA-HM1 phage exhibited a higher deposition efficiency than smaller (30 nm) and less hydrophobic (CA = 52°) H6/1 phages. Interestingly however, both marine phages showed lower retention than terrestrial T4 phages exhibiting similar characteristics than HM1. We conclude that marine phages have a high potential for the use as sensitive tracers in terrestrial habitats with their surface properties playing a crucial role for their transport.

## BIOSORPTION OF CARCINOGENIC PENTACHLOROPHENOL FROM AQUEOUS SOLUTIONS BY FUNGUS *ASPERGILLUS NIGER* BIOMASS

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### Background

Pentachlorophenol (PCP) is a hazardous agent in water and soil that poses a significant risk to human health. The toxicity associated with PCP occurs in low doses and mainly affect liver, kidney and central nervous system.

### Objectives

The purpose of this study was to investigate the biosorption of PCP on *Aspergillus niger* biomass as a method for removal of PCP from aqueous solutions.

### Methods

The modified *A. niger* biomass with NaOH was used to adsorb the PCP. The impacts of various experimental parameters such as initial PCP concentration, pH of solution, contact time, and biomass dosage were investigated and optimal conditions were identified. The experimental data were evaluated by various isotherm and kinetic models.

### Conclusions

The correlation of contact time, pH and initial concentration with the biosorption of PCP by *A. niger* biomass was statistically significant ( $P < 0.001$ ). The PCP removal increased with decreasing of solution pH and maximum adsorption was obtained at the pH 3. The equilibrium sorption capacity was increased by increasing initial PCP concentration from 10 to 40 mg/l of 4.23 to 11.65 mg/g, while the PCP removal efficiency decreased from 87 to 55%. Both Langmuir and Freundlich isotherms well described adsorption equilibrium of PCP on *A. niger* biomass. For all cases, the correlation coefficients for the second order kinetic model were close to one.

*A. niger* biomass can be used to reduce the toxicity of aqueous solutions containing PCP in acidic pH conditions. PCP concentration in water and soil poses a great hazard to human health, so its removal by fungi is applicable and commercial.

**FEMS-2716**

**Environmental microbiology - 2**

## **CULTURE-INDEPENDENT APPROACH FOR THE CHARACTERIZATION OF THE BACTERIAL COMMUNITY IN HAEMODIALYSIS WATER**

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### **Background**

The microbiological purity of haemodialysis water and dialysis fluid is crucial in the treatment of patients with chronic renal insufficiency. The presence of bacteria and bacterial-derived products (*i.e.*, endotoxins, sphingolipids, oligonucleotides) can contribute to silent chronic inflammation, secondary amyloidosis, pyrogenic reactions and anemia.

### **Objectives**

The main objective was to characterize the microbial community present in the haemodialysis water used to reconstitute the dialysis fluid at the Hospital Universitario Son Espases (Majorca, Spain). Bacterial community was assessed in samples obtained in November 2012 and 2013 to evaluate the evolution of the species colonizing these pure waters.

### **Methods**

Dialysis water was filtered through 0.22 µm pore size filters. DNA was extracted from the filters, purified and was amplified with two different set of primers: i) 16S rDNA universal primers and ii) selective *Pseudomonas* primers designed for the  $\sigma$ -subunit of the RNA polymerase sequence (*rpoD* gene). Amplicons were pyrosequenced with a 454 System+ GS FLX platform from Roche. Reads obtained were analyzed in order to assess the phylogenetic identification of bacteria present in the community.

### **Conclusions**

The bacterial community present in the dialysis water is very complex, with microorganisms highly diverse and adapted to the oligotrophic habitats, not usually detected routinely in clinical microbiology laboratories. This community is maintained in the two years period of this study. Based on the 16S rRNA gene pyrosequencing analysis the  $\alpha$ -*Proteobacteria* class is the most abundant. The pyrosequencing of the *rpoD* amplicon showed a high number of putative novel species of *Pseudomonas* showing a high diversity still not described.



**FEMS-2515**

**Environmental microbiology - 2**

## **ISOLATION, PCR IDENTIFICATION OF DIFFERENT BACTERIAL GROUPS AND PHYLOGENETIC CLASSIFICATION**

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### **Background**

Because of their massive utilization, hydrocarbons are major pollutants of soils and aquifers. Biodegradation is a key aspect of the fate of pollutants in the environment.

### **Objectives**

It is based on the Gas Chromatographic and Thin Layer Chromatography (TLC) coupled with a Flame Ionization Detector (FID) analysis of hydrocarbons, after incubation in optimal conditions of major compounds in crude oil (saturates and aromatics fractions).

### **Methods**

Using a method of gene sequencing 16S rDNA, the identification of bacteria adapted to soil was determined. Based on nearly full length 16S rRNA gene sequencing analysis, a phylogenetic trees was constructed. Capacity of degradation of selected bacteria and the presence or absence of functional genes coding for the initial oxidation of hydrocarbons (*alkB*, *nahAc*, *nidA*) were studied.

### **Conclusions**

The results indicate that the *Gamma-Proteobacteria* group was the main actor of this degradation. *Pseudomonas* sp, specific bacterial group suitable for petroleum hydrocarbon pollution has the ability to metabolize (single culture) a high mass residues, Pristane (*n*-C19) at 35.11% and Benzo[a]Pyrene (*n*-C20) at 33.93% and co-metabolize (in consortium) fractions in petroleum-hydrocarbon, 50.44% of saturates and 30.42% of aromatics compounds. Biodegradation efficiency of soil microflorae depended on the specific metabolic pathways of some microorganisms and on the cooperation within microbial population. This study provides a better understanding of the adaptation of bacteria inhabiting polluted environments and for developing and implanting adequate bio-strategies in the future to enhance oil in contaminated soil and the biotreatment of oily waste water in refineries.





**FEMS-0635**

**Environmental microbiology - 2**

**EXTRACTIVE MEMBRANE BIOFILM REACTORS (EMBFR) FOR BIOREMEDIATION OF METHYL TERT-BUTYL ETHER CONTAMINATED GROUNDWATER: A LAB-SCALE STUDY.**

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**Background**

Among the strategies developed for contaminated groundwater bioremediation, those based on the use of bacteria adhering to inert supports and established as biofilms, have gained great importance in this field. The EMBFR technology offers productive solutions for the removal of volatile compounds like methyl *tert*-butyl ether (MTBE) and other contaminants from water bodies. The EMBFR is based on extractive semipermeable (silicon) membranes through which the contaminants migrate to the biological compartment in which microorganisms with MTBE biotransformation and/or mineralization capacities grow in mineral salts medium, forming a catalytic biofilm on the membrane surface.

**Objectives**

The objective of this study was to assess the use of three bacterial strains (*Paenibacillus* sp. SH7 CECT 8558, *Agrobacterium* sp. MS2 CECT 8557, and *Rhodococcus ruber* EE6 CECT 8612) previously isolated from gasoline contaminated environments, and used as inoculum in a lab-scale EMBFR running for 28 days under aerobic conditions.

**Methods**

Three different retention times (1h, 6 h, and 12 h) were employed. Degradation rates were determined daily by gas chromatography - mass spectrometry, and the biofilm established by the bacterial strains on the semipermeable membrane was observed by FESEM at the end of each experiment. Acute toxicity of the effluents was determinate by Microtox<sup>®</sup> assay (EC<sub>50</sub>).

**Conclusions**

The results achieved from MTBE degradation, biofilm formation and toxicity analysis showed that these bacterial strains could be excellent candidates for use as selective inocula in EMBFR technology for MTBE bioremediation.

**FEMS-3123**

**Environmental microbiology - 2**

**DIARRHEAL BACTERIAL PATHOGENS ARE FREQUENT IN THE CHOQUEYAPU RIVER IN BOLIVIA, AND POSE A RISK OF TRANSMISSION TO FRESH PRODUCE BY CONTAMINATED IRRIGATION WATER**

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**Background**

Water borne diarrheal pathogens might accumulate in river water and present a potential contamination risk of drinking and irrigation water.

**Objectives**

In this study, we show how pathogens can accumulate and be transported in the river Choqueyapu in La Paz and contaminate fresh produce irrigated by river water.

**Methods**

Using qPCR absolute quantification methods, we found a seasonal and spatial variation in the number of pathogen gene copies per ml of river water with a high peak of *Campylobacter* *ssp.* that contaminated otherwise clean water in a watershed at the Altiplano plateau, after the peak of the rainy season in February 2014. High levels of *Campylobacter*, *Salmonella* and enterotoxigenic *E. coli* (ETEC) were subsequently isolated in sampling points downstream along the river and finally identified in vegetables rinse water.

**Conclusions**

These results indicate that distant water contaminations may be transported all the way to the agricultural area. In addition, we found that ETEC carrying the heat labile toxin (LT) alone was much more prevalent in water than other ETEC carrying the heat stable toxin (ST). Hence ETEC with different toxin profiles might have different reproduction and survival ability in water. The results show that the absolute levels of several diarrheal pathogens are high in contaminated river water in the central part of La Paz and although this water is not accessible by humans at this spot several of the pathogens end up in the downstream river water used for irrigation of crops where they might contaminate vegetables.

**FEMS-1659**

**Environmental microbiology - 2**

## **STUDY ON O, M, P-XYLENE DEGRADATION AND ITS RELATED GENES IN RHODOCOCCLUS SPP. J7**

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### **Background**

Xylene, an aromatic hydrocarbon, can be easily found in gasoline contaminated soil and composed of three isomers [ortho (o-), meta (m-) and para (p-) xylene]. Many bacterial strains have been reported to grow on omp-xylene isomers, but few studies have been performed to degrade them simultaneously. Xylene is known for its wide usage in tissue processing, staining and cover slipping and can find easily in ground water. Due to volatile property of xylene isomers, they can spread easily from groundwater to the air.

### **Objectives**

Once human is exposures to xylene, central nervous system could be damaged. So we tried to decrease amount of xylene emitted from factory using soil bacteria.

### **Methods**

Strain J7, belong to the genus *Rhodococcus* was isolated from total petroleum hydrocarbon contaminated site in Korea. Xylene degradation ability was evaluated using 90ppm of xylene mixture (30 mg/L individually) and analyzed by Agilent GC 6890N with Flame ionization detector (FID). The metabolites are analyzed using GC-MS and the pathway of degradation was construed. Target was amplified by PCR using genomic DNA and PCR products were sequenced at Marcrogen (Korea) and compared with other reference genes.

### **Conclusions**

From this study, we confirmed that strain J7 can degrade o, m, and p-xylene at the same time and based on the data, we have constructed that it follows TOL pathway with high efficiency. The catechol-1,2-dioxygenase gene was amplified and conforming that it followed ortho-cleavage pathway.

**FEMS-0759**

**Environmental microbiology - 2**

**KERATIN AZURE AND FEATHER DEGRADATION IN SOIL BY SOME FUNGI  
USING SINGLY AND IN COMBINATION**

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**Keratin azure and feather degradation in soil by some fungi using singly and in  
combination**

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**Abstract**

The large amount of keratinous waste produced and their localized accumulation create a serious disposal problem leading to environmental pollution. Keratin in forms of animal waste such as hairs, nails, horns, hooves and feathers ultimately comes to soil and if it remains there make pathogenic reservoirs because it is degraded by fungi which have pathogenic potential. On the other hand keratin when degraded by fungi nitrogen is slowly released in the soil and may be utilized by plants as manure. Keratinophilic fungi frequently occurring in soil were selected for hen feather and keratin azure degradation individually and in combination in different sets in soil. Five selected fungi *Acremonium implicatum*, *Chrysosporium queenslandicum*, *Chrysosporium pannicola*, *Malbranchea pulchella* and *Verticillium lecanii*, when used in combination with *Chrysosporium keratinophilum* showed feather and keratin azure degradation. Further feather and keratin azure degradation was monitored in soil when these were used individually and in combination with *C. keratinophilum* and almost above 90% degradation was recorded. The determination of amount of keratin degradation in soil by using individual fungus and fungi in combination is of immense importance in order to find out how much keratin is degraded in soil. The measurement of feather and keratin azure degradation in soil by using five fungi singly and in combination with *C. keratinophilum* presented here for the first time.



**FEMS-2820**

**Environmental microbiology - 2**

**LOW BIOMASS DNA AND RNA EXTRACTION IMPROVES BY USING G2 BLOCKINGREAGENT WITHOUT ANY BIAS IN METAGENOMC ANALYSIS**

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**Background**

Low biomass samples can be extremely challenging when extracting DNA and RNA. Many scientists have been adding various sources of RNA or DNA to the samples to aid a high recovery, however this makes the samples only useable for specifically directed analysis, while metagenomic analysis is hampered.

**Objectives**

A new product G2 that are shown to improve DNA and RNA yield from low biomass samples are tested for use in metagenomic analysis as well as if several labs can repeat the results in a ring test.

**Methods**

We have included analysis of influence of G2 on both full genome sequencing and various amplicon sequencing techniques. Further a Q-PCR analysis was performed after 12 laboratories in a ring test were applying G2 to one sampletype.

**Conclusions**

G2 was shown to be free of contaminating DNA both using 16SrDNA based Q-PCR and amplicon sequencing, further a metagenomic analysis clearly indicated that no traces of the original DNA could be found in a metagenome analysis. The ringtest showed that the participating laboratories all had a higher yield using G2 but also that the participating laboratories did perform very different.

**FEMS-2972**

**Environmental microbiology - 2**

**ANTIMICROBIAL ACTIVITIES AND BIOLOGICAL DIVERSITY OF CULTURABLE ACTINOBACTERIA ISOLATED FROM FRESHWATER FISH GUT MICROBIOTA**

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**Background**

Terrestrial microorganisms, especially those from the order Actinomycetales, formed the backbone for the golden age of antibiotic discovery, and are the source of the vast majority of FDA-approved antimicrobial agents.

**Objectives**

In this study, the antimicrobial activity and diversity of genes associated with secondary-metabolite biosynthesis of Actinobacteria isolated from fresh fish gut microbiota namely *Schizothorax zarudnyi* and *Schizocypris altidorsalis* were investigated.

**Methods**

Strains of six groups of fish and human pathogenic bacteria were selected and all the isolated actinomycetes were tested for their antibacterial activity against the pathogenic bacteria. The antagonistic activity of actinomycetes was tested by the perpendicular cross streak method.

The PCR-based approach was applied to detect the presence of three important biosynthetic genes including polyketide synthases (PKS-I and PKS-II), non-ribosomal peptide synthetase (NRPS). The PCR amplicons and their origins were further confirmed by sequencing.

**Conclusions**

According to results all isolates possess at least two types of the investigated biosynthetic genes. This study demonstrates the significant diversity of genes associated with secondary-metabolite biosynthesis of Actinobacteria in the fish gut microbiota and its potential to produce biologically active compounds.



**FEMS-1096**

**Environmental microbiology - 2**

**EFFECTS OF NITROGEN FERTILIZER ON NITRITE-DEPENDENT ANAEROBIC METHANE OXIDATION BACTERIA IN A FLOODED RICE PADDY**

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**Background**

Nitrite-dependent anaerobic methane oxidation (n-damo) may be an important process in the biogeochemical cycles of carbon and nitrogen. However, the inquiry of n-damo bacteria in rice paddy is rarely explored.

**Objectives**

The aim of this work was to investigate the effects of nitrogen fertilizer on the communities of n-damo bacteria in a flooded rice paddy.

**Methods**

Different amounts of urea (0, 45, 90, and 180 kg ha<sup>-1</sup>) were applied to a flooded rice paddy transplanted with a rice cultivar (*Oryza sativa*) and soil cores were sampled after 15 days of third urea application. The soil cores were divided into top (0–5 cm), middle (10–15 cm), and bottom (20–25 cm) layers and the diversity and abundance of n-damo were investigated using T-RFLP (Terminal-Restriction Fragment Length Polymorphism) and quantitative PCR approaches, respectively. A clone library was constructed for the T-RFLP analyses of 16S rRNA gene fragments of n-damo. DNA samples were retrieved from the soil core samples and the abundances and diversities of n-damo were investigated using T-RFLP.

**Conclusions**

Abundance of n-damo in a flooded rice paddy was not significantly different although different amounts of urea were applied. However, the phylogenetic analysis of *Methylobacterium oxyfera*-like 16S rRNA gene sequences revealed that n-damo was a little diverse depending on urea application and soil depths. Sequences of Top 90 soil sample fell into just one group, while sequences of Bottom 180 were classified into three groups. In addition, the diversity analysis of n-damo in response to different amounts of urea will be performed and discussed.

**FEMS-2881**

**Environmental microbiology - 2**

**MICROFLUIDIC CHIP-ASSISTED DNA PREPARATION FROM ENVIRONMENTAL SAMPLES SPIKED WITH FRANCISELLA TULARENSIS AND BACILLUS THURINGIENSIS**

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**Background**

The detection of bacteria from environmental samples using nucleic acid amplification depends on efficient DNA extraction and purification to remove PCR Inhibitors.

**Objectives**

DNA extraction and purification from spiked air, natural water, swab and soil samples was performed using a microfluidic chip device. A protocol was established that was suitable for the detection of two representative bacteria, *Francisella (F.) tularensis* (Gram-negative) and *Bacillus (B.) thuringiensis* (Gram-positive).

**Methods**

The procedure is composed of thermal, chemical and enzymatic lysis methods combined with on-chip DNA purification using paramagnetic particles for reversible DNA attachment. Polymeric microfluidic chips with rhombic cavities were mounted on a device with a regulated heating unit and a movable magnet that enables particle mixing for careful washing and elution. Serial 10-fold dilutions of the bacteria suspensions were tested. DNA preparations were analysed by real-time PCR assays to determine the detection limits that can be achieved.

**Conclusions**

Detection of both species of bacteria was possible from all tested matrices. The detection limits in air samples corresponded to 7 GE/10 l for *F. tularensis* and 0.7 GE/10 l for *B. thuringiensis*. In river water the limits were 10<sup>2</sup> GE/ml *B. thuringiensis* and 10<sup>3</sup> GE/ml *F. tularensis*. The achieved detection limits for soil samples corresponded to 2.7·10<sup>3</sup> GE/mg *F. tularensis* and 2.7·10<sup>2</sup> GE/mg *B. thuringiensis* and

with swab samples  $4 \cdot 10^3$  GE/cm<sup>2</sup> *F. tularensis* and  $4 \cdot 10^2$  GE/cm<sup>2</sup> *B. thuringiensis* were detected. Future developments will focus on the development of integrated lab-on-a-chip system combining sample preparation with on-chip PCR technologies.

**FEMS-0522**

**Environmental microbiology - 2**

**PLANT GROWTH PROMOTING RHIZOBACTERIAL EFFICACY IN COWPEA  
(VIGNA UNGUICULATA (L.) WALP.)**

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**Background**

Plant growth promoting rhizobacteria (PGPR) are known to influence the growth of the plant by various plant growth promoting activities such as Indole Acetic Acid (IAA) production, Phosphate solubilization activity, Ammonia production (NH<sub>3</sub>), Nitrogen fixation, Siderophore production and Hydrogen cyanide production (HCN). In search for the potential PGPR from the Rhizosphere soil of cowpea, a total of 16 rhizobacteria (B1-B6 & D1-D9) were isolated. By comparing the results two potential rhizobacteria (B6 & D4) have been selected for the further evaluation of Plant Growth Promoting (PGP) activity. These isolates biochemically characterized, B6 and D4 confirmed as *Pseudomonas* and *Bacillus* by 16SrRNA gene sequencing. *Invitro* and *Invivo* Nursery field studies revealed that 100 % seed germination rate using *Bacillus safensis* and 92% for *Pseudomonas aeruginosa* showing promising results. The study showed the characters of benefits to use these organisms as biofertilizers for sustainable agriculture to improve crop yield.

**Objectives**

This study was aimed to focus on the PGP activities of the two potential rhizobacteria and growth promotion

**Methods**

*IAA production (Lwin et al. 2012)*

*Siderophore production (Arnow 1987)*

*Phosphate Solubilization Activity (Qureshi et al. 2012)*

*Hydrogen Cyanide Production (Ahmad et al., 2008)*

*Ammonia Production (Cappucino and Sherman, 1992)*

*Nitrogen fixation activity (Lwin et al. 2012)*

*16S rRNA Sequencing*

*Seed germination rate and seedling vigor index (Ng LC et al. 2012)*

*Nursery pot experiment*

## **Conclusions**

Rhizobacteria isolated from the cowpea rhizosphere *Pseudomonas aeruginosa* VRKK1 and *Bacillus safensis* VRKK2 acts as the potential PGPR. *Pseudomonas aeruginosa* showed 100% germination rate, *Bacillus safensis* showed 92 % germination

**FEMS-2246**

**Environmental microbiology - 2**

**PROTEOMICS REVEALS OSMOTIC STRESS ADAPTATIONS OF SHEWANELLA BALTICA, PARACOCCLUS SP. AND FLAVOBACTERIUM SP. IN A UNIQUE ENVIRONMENT OF THE BALTIC SEA**

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**Background**

Baltic Sea constitutes a unique environment of brackish water conditions, with dynamic, regional shifts in salinity, mainly due to varying freshwater inflows.

**Objectives**

The goal of the study was to identify physiological and molecular strategies that allow Baltic Sea bacteria to confront this challenging habitat.

**Methods**

Three bacterial Baltic Sea representatives, identified as *Shewanella baltica*, *Flavobacterium* sp. and *Paracoccus* sp. on the basis of 16S rRNA sequencing and MALDI-TOF analyses, were cultured under controlled growth conditions in a chemostat system and analyzed with a combined approach of 2-DE, multiplex fluorescent staining of proteins in gels and LC-ESI-MS/MS and/or MALDI-TOF/TOF-driven protein identifications.

**Conclusions**

Tracking down of proteome dynamics in *Shewanella baltica*, *Flavobacterium* sp. and *Paracoccus* sp. in response to the salinity shifts characteristic of the Baltic Sea (2, 7 and 20‰) allowed the identification of a subset of proteins, both of the general stress response group and of other functional categories, differentially expressed under osmotic challenges tested. Wide variations in adaptations to osmotic stress were revealed in the three strains studied, with *Paracoccus* sp. requiring a significantly less

number of proteins to survive in the osmotic stress conditions in comparison to *Shewanella baltica* and *Flavobacterium sp.*

Taken together, our study uncovered well-coordinated and highly regulated protein inventories in the marine bacteria studied, with a large number of novel candidates for stress response players, thus laying a ground work for understanding of the complexity of microbial adaptive processes in the intriguing ecosystem of the Baltic Sea.

**FEMS-0728**

**Environmental microbiology - 2**

**BIOSORPTION OF CHROMIUM BY PSEUDOMONAS SP ISOLATED FROM SOIL CONTAMINATED WITH PETROLEUM OF KHOUZESTAN, IRAN**

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**Background**

In recent decades, the biological methods have been used for remove environmental contaminants such as heavy metals. Resistance to metals is important for this aim.

**Objectives**

The objective of this research is the isolation of chromium resistant strains.

**Methods**

Five samples of soil were collected under sterile conditions and transferred to laboratory immediately. Soil samples were homogenized and diluted up to 10<sup>-10</sup> with sterile normal saline. These samples were cultured in LB agar containing 5ppm of chromium in order to isolation of resistant strains. These strains were isolated and cultured in Macconkey agar for isolation of appropriate strains. Isolated bacteria were identified by biochemical tests. Then, the MIC test was used for screening of resistant strains. The best conditions of bacterial growth were found in the presence of chromium in various temperatures, rate of shaking and values of pH by spectrophotometry at 600nm in the overnight of cultivation. Metal adsorption test measured under optimal conditions by atomic absorption spectroscopy.

**Conclusions:** From total of 24 strains of isolated Pseudomonads, 14 strains were resistant to chromium. The top strain (Mac2) has eliminated 35.60% of chromium from aqueous culture mediums in optimal conditions (pH: 8, Temp: 40°C and Shaking rate: 200rpm). The results were verified using statistical method named the general linear model. The contaminated zones should be clean, because heavy metals in various physicochemical forms have been considered as environmental pollutants. This bacterium can be used for studies of bioremediation of contaminated sites to chromium.



**FEMS-0941**

**Environmental microbiology - 2**

## **MICROORGANISMS IN CAVES: IMPORTANCE, DIVERSITY AND CONSERVATION**

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### **Background**

Harsh conditions and isolation lead to formation of unique microbial communities, making caves a promising yet poorly understood field for study of biodiversity. Studies of microbial communities in caves may provide insights of microbial relationships, biogeochemical processes, evolution and even functioning of extraterrestrial life. Furthermore, they can lead to the discovery of novel bioactive compounds and draw attention to the importance of cave conservation.

### **Objectives**

The goal of the project was to analyze the microbial communities of different caves and evaluate the impact of human activity within pristine and disturbed cave areas.

### **Methods**

Barcode pyrosequencing of 16S rRNA genes was used to investigate the microbial diversity of two oligotrophic Karabi Jaila (Crimea) caves and the world's deepest cave Krubera-Voronja (Caucasus).

### **Conclusions**

It was found that 12 % of 16S rRNA gene sequences from the more frequently visited areas of Crimean caves belonged to previously unclassified bacteria. In the samples of rarely visited branches the sequences belonging to unclassified bacteria comprised 46 %. On the other hand, only 5 % of sequences found in more pristine areas of Krubera-Voronja cave belonged to unclassified bacteria. 6 % of unclassified sequences were found in the main branch of the cave where human activity is particularly intense. Disturbed areas of all caves demonstrated broader diversity of known phyla as well as significantly higher abundance of sequences belonging to *Bacteroidetes*, which are known to be indicators of fecal pollution. The authors are thankful to the Lithuanian Science Council for the financial support (project No. MIP-005/2014).

**FEMS-1237**

**Environmental microbiology - 2**

## **IDENTIFICATIONS OF PROPER METHODS TO DETERMINE LEGIONELLA PNEUMOPHILA BACTERIA EXPOSED TO DIFFERENT ENVIRONMENTAL CONDITIONS**

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### **Background**

*Legionella* which is the agent of Legionnaires' disease in natural and man-made water systems. *Legionella* bacteria pass to the viable but non-culturable (VBNC) phase due to the environmental conditions such as temperature, pH and disinfectants. After examining under laboratory conditions the water samples containing *Legionella* bacteria, have been exposed to environmental factors, it was shown that those bacteria couldn't colony form medium, but this result does not mean that the bacteria are not present in a sample or an environment.

### **Objectives**

The aim of the present study is to detect the most suitable methods for the recovery of *L. pneumophila* bacteria exposed to different environmental conditions.

### **Methods**

In the current study, first of all, the detection limits of culture, fluorescent *in situ* hybridization (FISH) and semi-nested polymerase chain reaction (PCR) methods for different *L. pneumophila* concentrations ( $10^2$ - $10^{10}$  cell/L) was determined. Later, the recovery of *L. pneumophila* bacteria from the water samples including *L. pneumophila* bacteria that expose to different environmental conditions (temperature, pH and biocide) has been assessed by culture, FISH and semi-nested PCR methods.

### **Conclusions**

In the present study, the detection limit of *L. pneumophila* bacteria was determined as  $10^3$  cell /L for the culture method,  $10^5$  cell/L for FISH method and  $10^6$  cell /L for semi-nested PCR method. It was determined that FISH and semi-nested PCR methods are suitable methods for the recovery of *L. pneumophila* bacteria that are subject to different environmental conditions from the water samples.



**FEMS-1671**

**Environmental microbiology - 2**

**ANTIBIOTIC RESISTANCE PATTERN OF PSEUDOMONAS SPP. ISOLATED FROM THE TOTAL COURSE OF THE RIVER DANUBE**

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**Background**

This study was part of the Joint Danube Survey 3 (JDS3), the world's biggest river research expedition of its kind.

**Objectives**

The aim of the study was to determine the presence of acquired antibiotic resistance of different bacterial species with clinical impact.

**Methods**

During JDS3 180 water samples were taken over the total course of the river Danube. Samples were mixed with glycerol and stored at -70°C. For isolation five ml of the thawed samples were plated in 0.5 ml portions on *Pseudomonas* selective agar and incubated at 37°. The isolates were tested for species identification with mass spectrometry MALDI-TOF MS (Shimadzu, Japan). Resistance testing was performed as recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). To estimate the presence of carbapenemase modified hodge tests were performed with all isolates resistant to at least one carbapenem.

**Conclusions**

Up to now 496 *Pseudomonas* were isolated, 343 (67%) *Pseudomonas putida*, 120 (25%) *Pseudomonas fluorescens*, all other species were represented by less than 10 isolates but no *Pseudomonas aeruginosa* was observed. Most common resistance was against meropenem (20.4%/ 76 isolates) piperacillin/tazobactam (4.3%/16 isolates) and ciprofloxacin (4.3%/16 isolates). None of the antibiotics were susceptible in all tested strains. Resistances were present all over the tested sampling points with no significant changes downstream. One isolate (*Pseudomonas fluorescens*) was tested positive for the presence of a carbapenemase in modified hodge test. Although the isolated *Pseudomonas* species are clinically less frequent, they are a

possible reservoir for resistance acquisition for other species (especially *Pseudomonas aeruginosa*).

**FEMS-3134**

**Environmental microbiology - 2**

**SELECTION OF A SYNTHETIC SOIL MICROBIAL COMMUNITY FOR  
QUANTIFYING EFFECTS OF HYDRATION DYNAMICS ON COMMUNITY  
STRUCTURE AND DIVERSITY**

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**Background**

Soil bacteria play a central role in many ecosystem services such as formation of rhizosphere communities, nutrient transformation and global biogeochemical cycles. Although molecular techniques have uncovered the tremendous bacterial diversity in soil, the mechanisms that control the assembly, functioning and maintenance of complex microbial communities remain largely unknown.

**Objectives**

The study aims at linking dynamics of soil hydration conditions and related biophysical factors with variations in microbial composition and ecological functioning. Understanding factors that shape the dynamics of species abundance is essential to get an insight into the assembly of a microbial community, its functioning and response to induced changes in water availability, a key parameter of soil environments.

**Methods**

To assist with definitive community level observations we designed a synthetic microbial community comprised of 10 well-characterized bacterial species spanning a wide range of soil phyla to be inoculated onto model porous surfaces mimicking soil habitats. The experimental system consists of sand layer placed on porous ceramic surface connected to nutrient reservoir. Fluctuations in hydration conditions are induced by changing the reference nutrient reservoir level to simulate wetting-drying cycles. The activity and composition of the bacterial community is studied using 16S fingerprinting and quantitative PCR.

**Conclusions**

Spatial self-organization of microbial community is highly dependent on the hydration level of their habitat. Subjecting the synthetic community to a range of controlled physico-chemical conditions allows us systematic evaluation of the role of environmental fluctuations on changes in community composition.



**FEMS-2700**

**Environmental microbiology - 2**

**TRANSCRIPTOME ANALYSIS OF THE BALTIC SEA ISOLATES: SHEWANELLA BALTICA, PARACOCCLUS SP. AND FLAVOBACTERIUM SP. SHOWS THEIR SPECIFIC RESPONSE TO OSMOTIC STRESS.**

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**Background**

The Baltic Sea constitutes very specific environment. One of its characteristics are brackish water conditions that are caused by high water input from the surrounding lands and rivers. However, because of its characteristic geographical location, salinity levels change significantly in different parts of the Sea and can extend from 2, through 7 and even up to 20‰.

**Objectives**

The aim of our work is to assess which genes are essential for marine bacteria to adapt to the changes in salinity of their environment.

**Methods**

Three bacteria isolated from Baltic Sea were sequenced using Illumina HiSeq 2,000 platform and characterized as *Shewanella baltica*, *Paracoccus* sp. and *Flavobacterium* sp.. Then, they were cultured in a chemostat system in the minimal marine medium at various salinity conditions. During steady-state growth cells were harvested for RNA extraction and Illumina-based RNA-Seq reads were analyzed with DESeq2 software.

**Conclusions**

The transcriptome analysis of the three strains *Shewanella baltica*, *Paracoccus* sp. and *Flavobacterium* sp. at various salinity levels (bacteria cultured in 2, 7 and 20 ‰) resulted in identification of groups of genes responsible for bacterial survival in the



challenging environment of the Baltic Sea. Each strain is characterized by different response to osmolarity stress, underlying the importance of diverse categories of functional genes in this marine habitat.

**FEMS-0643**

**Environmental microbiology - 2**

## **FUNCTIONAL RESILIENCE OF MICROBIAL ECOSYSTEMS IN SOIL: HOW IMPORTANT IS A SPATIAL ANALYSIS?**

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### **Background**

Microbial life in soil is exposed to fluctuating environmental conditions influencing the performance of ecosystem services such as biodegradation.

### **Objectives**

However, as this environment is typically very heterogeneous, spatial aspects can be expected to play a major role for the ability to recover from a stress event.

### **Methods**

To determine key processes for functional resilience, scenarios with varying stress intensities were simulated within a microbial simulation model and the biodegradation rate in the recovery phase measured. Besides temporal monitoring, results were analyzed regarding spatial and mechanistic aspects. Parameters including microbial growth and dispersal rates were varied over a typical range to consider microorganisms with varying properties.

### **Conclusions**

Results of the mechanistic and spatial view show that key factors for functional recovery depend on stress intensity and the location of the observed habitats. The limiting factors near unstressed areas are spatial processes (motility and substrate diffusion), with increasing distance microbial growth becomes more important. To confirm this, we repeated the simulations including a dispersal network representing fungi in soil. The system benefits from an increased spatial performance due to higher bacterial mobility.

With these simulations we show the importance of spatial aspects even at the mm-scale for recovering after a severe stress event in a highly heterogeneous environment such as soil. In consequence a spatial-mechanistic view is necessary for examining the functional resilience as the temporal view alone could not have led to these conclusions.

Further research should explore the importance of a spatial view for quantifying functional resilience also after complex stress regimes.



**FEMS-0644**

**Environmental microbiology - 2**

**UNCOVERING STABILITY MECHANISMS IN MICROBIAL ECOSYSTEMS -  
COMBINING MICROCOSM EXPERIMENTS, COMPUTATIONAL MODELING AND  
ECOLOGICAL THEORY IN A MULTIDISCIPLINARY APPROACH**

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**Background**

Although bacterial degraders in soil are commonly exposed to fluctuating environmental conditions, the functional performance of the biodegradation processes can often be maintained by resistance and resilience mechanisms.

**Objectives**

However, there is still a gap in the mechanistic understanding of key factors contributing to the stability of such an ecosystem service

**Methods**

Therefore we developed an integrated approach combining microcosm experiments, simulation models and ecological theory to directly make use of the strengths of these disciplines. In a continuous interplay process, data, hypotheses, and central questions are exchanged between disciplines to initiate new experiments and models to ultimately identify buffer mechanisms and factors providing functional stability. We focus on drying and rewetting-cycles in soil ecosystems, which are a major abiotic driver for bacterial activity.

**Conclusions**

Functional recovery of the system was found to depend on different spatial processes in the computational model. In particular, bacterial motility is a prerequisite for biodegradation if either bacteria or substrate are heterogeneously distributed. Hence, laboratory experiments focussing on bacterial dispersal processes were conducted and confirmed this finding also for functional resistance. Obtained results will be incorporated into the model in the next step. Overall, the combination of computational modelling and laboratory experiments identified spatial processes as the main driving force for functional stability in the considered system, and has proved a powerful methodological approach.

**FEMS-1308**

**Environmental microbiology - 2**

**BIOEGO: DEVELOPMENT OF A FUNGAL-BACTERIAL CONSORTIUM-BASED SOIL INOCULANT FOR PLANT GROWTH PROMOTION AND BIOCONTROL**

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**Background**

There is an emerging need for environment-friendly soil inoculants with favorable effects on crop plants.

**Objectives**

We aimed to develop BioeGO, a fungal-bacterial consortium-based soil inoculant ensuring increased nitrogen fixation, phosphorous mobilization, stem degradation and humus production on the treated fields and providing protective effects against phytopathogenic fungi.

**Methods**

Nitrogen-fixing component was selected from bacteria based on growth capabilities in nitrogen-free medium. Phosphorous-mobilizing and stem-degrading component was selected from *Trichoderma* strains based on cellulase- and phosphatase-producing abilities. Humus-producing component was selected from bacteria based on peroxidase-producing abilities, while biocontrol component was selected from *Trichoderma* strains based on *in vitro* antagonism towards phytopathogenic fungi.

**Conclusions**

An *Azotobacter vinelandii* strain is the nitrogen-fixing component of the soil inoculant with the potential to provide excess nitrogen for crops. Phosphorous mobilization and stem degradation are ensured by a *Trichoderma harzianum* strain producing cellulose-degrading enzymes in the absence of stem residues, while this ability is increased 10-15 fold in the presence of grinded maize stem. It also produces large amounts of enzymes liberating organically bound phosphorous. A *Streptomyces albus* strain with excellent peroxidase-producing abilities was selected as the humus-producing component, while a *Trichoderma asperellum* isolate with outstanding antagonistic abilities towards *Fusarium*, *Phoma*, *Alternaria*, *Botrytis* and *Rhizoctonia*

strains is the biocontrol component of BioeGO. Microbial components of the consortium do not have negative effects on each other, thereby all beneficial effects can occur simultaneously, complementing each other. This research was supported by grant TÁMOP-4.1.1.C-12/1/KONV-2012-0012. Csaba Vágvolgyi thanks the visiting professor program, Deanship of Scientific Research, King Saud University, Riyadh.

**FEMS-3126**

**Environmental microbiology - 2**

## **CHARACTERIZATION OF POTENTIAL AEROSOLIC IN ACTIVITY OF FUNGAL SPECIES**

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### **Background**

Biological aerosol particles are ubiquitous in the atmosphere. Recent findings identified a pool of these particles as potential ice nucleators (IN), which are capable of catalyzing ice formation at relatively warm subfreezing temperatures and thus impact cloud formation and precipitation. Some fungal species have been identified as ice nucleation active (INA) e. g., the ice nucleation capacity of *Fusarium acuminatum* was described already some decades ago but recently more species were discovered to possess IN activity (e.g., *Mortierella alpina*). For fungal IN activity, a protein or at least a proteinaceous compound is hypothesized to be responsible.

### **Objectives**

The aim of the study is to analyze the ability to release cell-free IN into the atmosphere. Therefore, the IN machinery of fungal species is characterized by analytical and molecular biological methods as well as chamber experiments with the ultimate goal to clarify their potential impact on atmospheric cloud formation and water precipitation.

### **Methods**

In this study, liquid chromatography and mass spectrometry is performed to identify proteinaceous compounds of fungal IN. A specific IN trap chamber (IN-TC) is designed to analyze the aerosolic transport capacity of fungal IN. For testing functional IN activity a customized droplet freezing assay is used.

### **Conclusions**

Functional IN active fractions of fungal protein surface extractions can be identified by liquid chromatography and SDS-PAGE. Preliminary IN-TC results show a successful proof of principle suggesting the chamber is capable of collecting aerosolic IN generated of fungal washing water.





**FEMS-2963**

**Environmental microbiology - 2**

## **DEVELOPMENT OF HEAVY METAL ION BIOSENSORS USING SYNTHETIC GENETIC CIRCUITS**

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### **Background**

We previously determined the genome of *Bacillus oceanisediminis* 2691 that was isolated from marine sediment of the South Korean coast and found that many genes encoded heavy metal resistance and efflux systems in the genome. Genes encoding putative cadmium efflux pumps, arsenic efflux pumps, a chromate transporter, and lead-, cadmium-, zinc-, and mercury-transporting ATPases were found in the genome, which are apparently regulated by CadC homologous repressor-type transcription factors.

### **Objectives**

We attempted to develop heavy metal ion biosensors using synthetic genetic circuits with *cadC* genes.

### **Methods**

It was confirmed the transcriptional modulations of CadC-controlled genes in response to various heavy metals in *B. oceanisediminis* 2691 using real-time PCR. Six *cadC* promoter-operator-structural genes were transcriptionally fused with *egfp* gene to make recombinant *E. coli* cells. A variety of heavy metals were treated and specific fluorescence intensities were measured.

### **Conclusions**

Taken together, the results showed that CadC proteins specifically respond to heavy metals and may play separate roles in heavy metal resistances, which have been evolved in the heavy metal abundant marine sediment milieu. In biotechnological aspects, CadC-controlled transcriptional modules could be used in the development of harmful heavy metal bio-sensor.



## CHARACTERIZATION OF COD REMOVAL, ELECTRICITY GENERATION AND MICROBIAL COMMUNITY IN MICROBIAL FUEL CELLS TREATING MOLASSES WASTEWATER

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### Background

Wastewater microbial fuel cells (MFCs) have advantages in treatment wastewater and electricity production simultaneously. Molasses wastewater (MW) is one of suitable feedstock for wastewater MFCs.

### Objectives

In this study, electricity generation, COD removal, and microbial communities were investigated using 3-types of MFCs which were single-chamber MFCs without proton exchange membrane (PEM) and with PEM (AC-MFCs and ACM-MFCs, respectively) and two-chamber MFCs (H-MFCs).

### Methods

Diluted MW (10,000 mg·L<sup>-1</sup> COD) was continuously fed into the MFCs. Reactor performances in the single-chamber MFCs were similar, indicating that PEM couldn't enhance the reactor performances. COD removal in the single-chamber MFCs (90%) was higher than in the H-MFCs (50%). However, current density in the H-MFCs (80 mA·m<sup>-2</sup>) was 1.4 times of that in the single-chamber MFCs (57 mA·m<sup>-2</sup>). Power density in the H-MFCs (17 mW·m<sup>-2</sup>) was 2.2 times that in the single-chamber MFCs (7.7 mW·m<sup>-2</sup>). Microbial communities of the MFCs were examined using 16S rRNA-iron torrent sequencing. For bacterial community, *Clostridium* (7.6%) and *Geobacter* (4.2) were abundant in the AC-MFCs, and *Clostridium* (7.6%) and *Desulfovibrio* (5.1%) in the ACM-MFCs. Bacterial community in the H-MFCs was different with the single-chamber MFCs. *Pseudomonas* (12.9%) was dominated in the H-MFCs, followed by *Clostridium* (7.0%) and *Geobacter* (2.9%). For archaeal community, *Methanothrix* (96.4%) was remarkably dominated in the single-chamber MFCs. However, *Methanobacterium* (35.1%) and *Methanosarcina* (28.3%) were abundant in the H-MFCs.

### Conclusions

These results suggest that chamber design is a key factor to influence on COD removal and electricity generation in the MFCs treating MW.

**FEMS-2932**

**Environmental microbiology - 2**

**GENOMIC INSIGHTS INTO THE CATABOLIC VERSATILITY OF RHODOCOCCLUS SP. STRAIN P52 TOWARDS ENVIRONMENTAL POLLUTANTS**

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**Background**

*Rhodococcus* sp. strain p52, an isolated dioxin-degrader, can aerobically utilize a variety of aromatic compounds, such as dibenzofuran, dibenzo-p-dioxin, 2-chlorodibenzofuran, 2,8-dichlorodibenzofuran, dibenzothiophene, biphenyl, naphthalene, fluorene, phenanthrene, and anthracene. Degradation of dioxin by strain p52 involves two dioxygenases, DbfA and DfdA, encoded by genes on two plasmids. Additionally, strain p52 can use linear alkanes (tetradecane, tetracosane, and dotriacontane) and branched alkane (pristane) as sole carbon and energy sources.

**Objectives**

The objective of the present study is to gain insight into the underlying genetic information for the catabolic versatility, and biodegradation potential of strain p52.

**Methods**

Complete nucleotide sequence of the strain p52 genome was sequenced with the PacBio RS II system. The genome was annotated by BLAST against database, including KEGG, COG, SwissProt, TrEMBL and NR. The predicted genes involved in degradation of aromatic compounds and alkanes were tested for their transcriptional activity. And the function of the genes was confirmed by heterologous expression in recombinant hosts.

**Conclusions**

Co-presence of catabolic plasmids and multiple catabolic genes, as well as genes involved in chemotaxis, solvent tolerance, and transportation in the strain favors its catabolic ability and environmental adaptation. The present study provides insights into the bioremediation potential of strain p52.

**FEMS-0818**

**Environmental microbiology - 2**

### **BIODEGRADATION OF P-NITROPHENOL BY DIFFERENT MIXED CULTURES**

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#### **Background**

One of the most important environmental pollutant among nitroaromatic compounds is *p*-nitrophenol (4-nitrophenol, PNP). It is used in the manufacture of a wide range of products and is the intermediate of some organophosphorus pesticide degradation (1). PNP has been classified as a priority pollutant by United States Environmental Protection Agency (EPA), and its concentration in natural waters is restricted to less than 10 ng/L (2).

#### **Objectives**

The aim of this study was to investigate the biodegradation of PNP by three different mixed cultures (TUS, AM and KOV) isolated from contaminated soil and sediment.

#### **Methods**

The samples were inoculated into minimal medium with increasing PNP concentrations. The biodegradation process was monitored by UV spectroscopy. The toxicity of PNP solution and PNP degradation intermediers to luminescent bacteria *Aliivibrio fischeri* was evaluated according the ISO 11348-3.

#### **Conclusions**

The mixed culture TUS showed the greatest biodegradation potential. Mixed microbial population was capable of degrading up to 100 mg/L of PNP as a sole carbon and nitrogen source. Toxicity was significantly reduced after the degradation process.

#### **References:**

1. Zhang J et al. Journal of Hazardous Materials 163 (2009) 723.
2. Zohar S et al. International Biodeterioration and Biodegradation 84 (2013) 80.

**FEMS-1603**

**Environmental microbiology - 2**

**CHARACTERIZATION OF A THERMO-STABLE BIOFLOCCULANT PRODUCED BY METHYLOBACTERIUM SP**

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**Background**

Chemical and organic synthetic flocculants are, currently, predominantly employed in different industrial processes such as wastewater treatment and drinking water purification. The use of bioflocculants is desirable as most chemical and organic synthetic flocculants have been shown to possess deleterious effects to human health and, also, are recalcitrant to biodegradation thus, polluting the environment.

**Objectives**

This study assessed the bioflocculant production potential of *Methylobacterium* sp. Obi isolated from Tyume River in the Eastern Cape Province, South Africa.

**Methods**

*Methylobacterium* sp. Obi (accession number HQ537130) identified through 16S rDNA and sequence BLAST analysis was evaluated for bioflocculant production and the physicochemical properties optimal for the production of bioflocculant was determined using standard technique. The bioflocculant was characterized for novelty using FTIR and thermo-gravimetric analysis.

**Conclusions**

Culture conditions for optimal production of the bioflocculant included the following: glucose as carbon source; complex nitrogen source of urea, yeast extract and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>; inoculum size of 1% (v/v); initial pH 10; and 1% Ca<sup>2+</sup>. Maximum flocculating activity of 90% was attained after 36 hr of cultivation with the bioflocculant exhibiting thermal stability up to 100°C. Optimal culture conditions resulted in purified bioflocculant yield of 4.61 g/l. Optimal dose for kaolin suspension clarification 0.3 mg/ml in the presence of 1% Ca<sup>2+</sup>. Fourier transform infrared (FTIR) spectrum of the purified bioflocculant showed the presence of carboxyl, amino and hydroxyl groups known to be critical in the flocculation process. Thermo gravimetric analysis revealed that the produced bioflocculant was thermo stable.



**FEMS-2553**

**Environmental microbiology - 2**

**COMMUNITY STRUCTURE OF BENEFICIAL RHIZOBACTERIA IN APPLE ORCHARDS OF TRANS HIMALAYAN REGION AS INFLUENCED BY PHYSICAL GEOGRAPHY OF AN APPLE HILL FARM**

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**Background**

Over the years, diversity of beneficial rhizobacteria associated with agronomical/horticultural crops, especially the influence of geographical distribution (altitudinal variation) has been well documented. However, the influence of physical geography (eastern and western aspect of a hill farm), on the community structure of beneficial rhizobacterial population, irrespective of the altitudinal variation is unclear.

**Objectives**

The nutrient and health status of crops growing on two opposite geographical aspects of a hill farm could be influenced by presence or absence of beneficial rhizobacterial communities, with exceptional abilities of nutrient mobilization and pathogen antagonism.

**Methods**

In the present study we investigated rhizobacterial population associated with apple trees (four different apple cultivars) grown on hill farms at similar altitudes on two opposite geographical aspects. In the present study, a total of 259 rhizobacteria were isolated from the rhizosphere of apple trees, of which 50 were selected and further examined on the basis of their abilities of nutrient mobilization and pathogen antagonism. Beneficial rhizobacterial diversity analyzed using molecular analysis (16S rDNA-ARDRA) and supported by statistical tools revealed the presence of dominant siderophore producing bacterial populations (nutrient mobilizers and plant pathogen antagonists) on the eastern aspect of hill farm.

**Conclusions**

Results from microbial diversity study correlated with the survey analysis, for the presence of 40-60% of diseased apple trees and high (80-90%) non-survivability of re-planted apple plants on the western aspect of hill farm, as compared to 10-15%



diseased apple trees and low problem (15-20%) of survivability of re-planted apple plants on eastern aspect.

**FEMS-1201**

**Environmental microbiology - 2**

**ENTEROCOCCUS FAECIUM REDUCED MALODOROUS COMPOUNDS IN SWINE MANURE SLURRY IN VITRO AND IN CONFINEMENT HOUSES**

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**Background**

Reducing malodorous compounds are still swine producers' problem.

**Objectives**

This study evaluated different bacteria for their ability to reduce malodorous compounds.

**Methods**

Previously isolated and identified bacteria with 99% similarities were used in this study. These were *Enterococcus faecium*(M1), *E. faecalis*(M2), *Acetobacter tropicalis*(M3) and *Bacillus subtilis* subsp. *subtilis*(M4). The bacteria were tested using *in vitro* technique (serum bottles and plastic boxes) and the best bacterium was selected and used as spray in the confinement pig houses.

**Conclusions**

In serum bottles technique, pH values were reduced (P<sub>3</sub>) and hydrogen sulfide (H<sub>2</sub>S) gas decreased (P and M4 from 12 to 24h while opposite was observed in NH<sub>4</sub><sup>+</sup>-N concentration in the liquid phase. Total volatile fatty acid, histamine, methylamine, ethylamine, putrescine and cadaverine as well as total biogenic amine concentration was lowest (P<sub>3</sub>, H<sub>2</sub>S, total mercaptans) and liquid NH<sub>4</sub><sup>+</sup>-N were comparatively lower (P and M4. M1 was selected and used for spray to confirm the odor reduction at different periods in the confinement pig houses. Odor compounds were lessened by 28.21%, 42.15% and 15.02% for NH<sub>3</sub>, H<sub>2</sub>S, and total amines, respectively up to 3 days after spraying. *E. faecium* can reduce malodorous compounds in swine manure slurry *in vitro* and in confinement houses.

**FEMS-1203**

**Environmental microbiology - 2**

**COMPARISON OF DIFFERENT BACTERIA ON REDUCING MALODOROUS COMPOUNDS USING IN VITRO SWINE FERMENTATION**

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**Background**

Bacteria play a significant role in odor production.

**Objectives**

This experiment was conducted to determine the appropriate bacteria for reducing malodorous compounds during fermentation in pig.

**Methods**

The bacteria were isolated and identified from rectum of pigs and were used in this study. They have 99-100% similarities to: *Enterococcus faecium* CWBI B1430(M1), *E. faecium* DSM 20477(M2), *E. faecalis*(M3), *Lactobacillus acidophilus*(M4), *L. plantarum*(M5), *Bacillus subtilis* ZHA10(M6), *Bacillus subtilis* XJASZB15(M7), *Acetobacter malorum*(M8), and *E. faecium* NRIC0114(M9). One hundred ml of fecal slurry was transferred to serum bottles containing 1g of soluble starch, inoculated with or without 1ml of different microbial cultures ( $1.0 \times 10^7$  CFU/ml) and incubated anaerobically for 12 and 24h.

**Conclusions**

Ammonia-nitrogen (NH<sub>3</sub>-N) contents (gas phase) were decreased rapidly from 12 to 24h but was not found in M7 and M9 at 24h while NH<sub>3</sub>-N contents (liquid phase) of M6 was significantly lowest after 24h. Hydrogen sulfide (H<sub>2</sub>S) contents (gas phase) were detected in M5, M6, M7, M8 and M9. Total biogenic amine was found lowest (*P**Enterococcus faecium* NRIC 0114(M9) is the best among the all of the treatments on the basis of toxic compound production.

**FEMS-1405**

**Environmental microbiology - 2**

## **GRAZING BY NEMATODES AFFECTS MAIZE RHIZOSPHERIC BACTERIA**

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### **Background**

Both mutualistic and pathogenic bacteria colonize the plant rhizosphere. Mutualists promote plant growth by providing nutrients or defense against pathogens. Nematodes and protozoa are also enriched in the rhizosphere, but little is known about the interactions of the grazing microorganisms with the bacterial community. The diversity of protozoans and nematodes in the corn rhizosphere has't been studied and will provide a basis for the further analysis of their grazing preferences and their impact on bacteria.

### **Objectives**

Identify and isolate protozoans and nematodes from the corn rhizosphere with metagenomic and culture-dependent approaches.

### **Methods**

The diversity of the eukaryotic microorganisms was assessed analyzing 18S rRNA gene sequences from DNA extracted from corn rhizosphere samples. Nematodes were cultured in Nematode Growth Medium (NGM) and identified as described above. Feeding preferences were assayed in Petri dishes with NGM medium in the presence of different bacteria. The effect of nematodes was tested by adding the nematode to roots of maize plants growing in flasks with vermiculite.

### **Conclusions**

The metagenomic analysis was performed with DNA samples from Huitzilac in the state of Morelos and in Amecameca in the state of Puebla. In cultures, the single nematode present was identified as *Oscheius tipulae*. This nematode has a preference for the bacterium *Rhizobium phaseoli* Ch24-10, and decreases the growth of corn plants in laboratory assays. However, in the presence of a group of selected root bacteria such as *Rhizobium phaseoli*, *Methylobacterium extorquens* and *Chromobacterium violaceum*, the growth of the corn plants was enhanced compared to control plants without the nematode.

**FEMS-0942**

**Environmental microbiology - 2**

## **VIBRIO VULNIFICUS EMPLOYS DIFFERENT DEFENCE MECHANISMS AGAINST PROTOZOAN GRAZING**

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### **Background**

*Vibrio vulnificus* is an opportunistic pathogen responsible for wound infections and septicæmia following ingestion of contaminated seafood, and has the highest reported mortality rate for seafood-related diseases. It is an autochthonous inhabitant of coastal marine environments where it is exposed to predation by heterotrophic protozoa.

### **Objectives**

Protozoan predation on bacteria acts as a selective force, leading to evolution of antiprotozoal mechanisms that may also function as virulence factors. This study investigated the correlation of the place of isolation, genotype and virulence potential with grazing resistance of *V. vulnificus*.

### **Methods**

Twenty two strains of *V. vulnificus* were exposed to grazing by *Tetrahymena pyriformis*, a filter feeding ciliate, and *Acanthamoeba castellanii*, a surface browsing amoeba in microtitre plates. *V. vulnificus* numbers in planktonic phase were determined by plate counts and photometry while biofilm biomass was assessed by crystal violet staining. The health and number of protozoa were determined by microscopy.

### **Conclusions**

Although there was no significant difference in grazing resistance between C-genotype (clinical origin) and E-genotype (environmental origin), an environmental and a clinical isolates exhibited unique grazing defence mechanisms against the protozoa: the oyster isolate showed toxicity against *T. pyriformis* while the blood

isolate employed biofilm and pellicle formation to escape predation. Furthermore, the role of capsular polysaccharide (CPS) in grazing resistance of *V. vulnificus* was examined; no statistically significant differences were found between the encapsulated, 'opaque' strains and the non-pathogenic non-encapsulated, 'translucent' strains. These results show that the defence mechanisms of different strains of *V. vulnificus* have evolved along different evolutionary pathways.

**FEMS-2080**

**Environmental microbiology - 2**

## **MICROBIAL COMMUNITY DYNAMISM DURING START-UP OF ANAEROBIC DIGESTER**

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### **Background**

The start-up of anaerobic digester has not been studied in detail from the microbiological point of view. Knowledge of microbiology process could help to determine whether an anaerobic digester has reached steady state, in the event that physicochemical data are not successful.

### **Objectives**

The objective is to determine the dynamism of the microbial community during the start-up of an anaerobic digester.

### **Methods**

The start-up of two-mesophilic anaerobic digester (fed with mix sludge) was studied through methane productivity, volatile suspended solids (biomass), along with other chemical variables. The inoculum was obtained from 'La Farfana', wastewater treatment plant located in Santiago, Chile.

DNA was extracted from samples of sludge operation days 0, 13, 25 and 50. The genes studied were: 16S (*Bacteria* and *Archaea*) through DGGE and 4 functional genes (Hydrolytic, Homoacetogenic, Methanogenic and Acidogenic) through real time PCR.

### **Conclusions**

The digester reached steady state after 30 days of operation, with productivity of 140 mL methane/ (Liter day) and 40% increase in biomass.

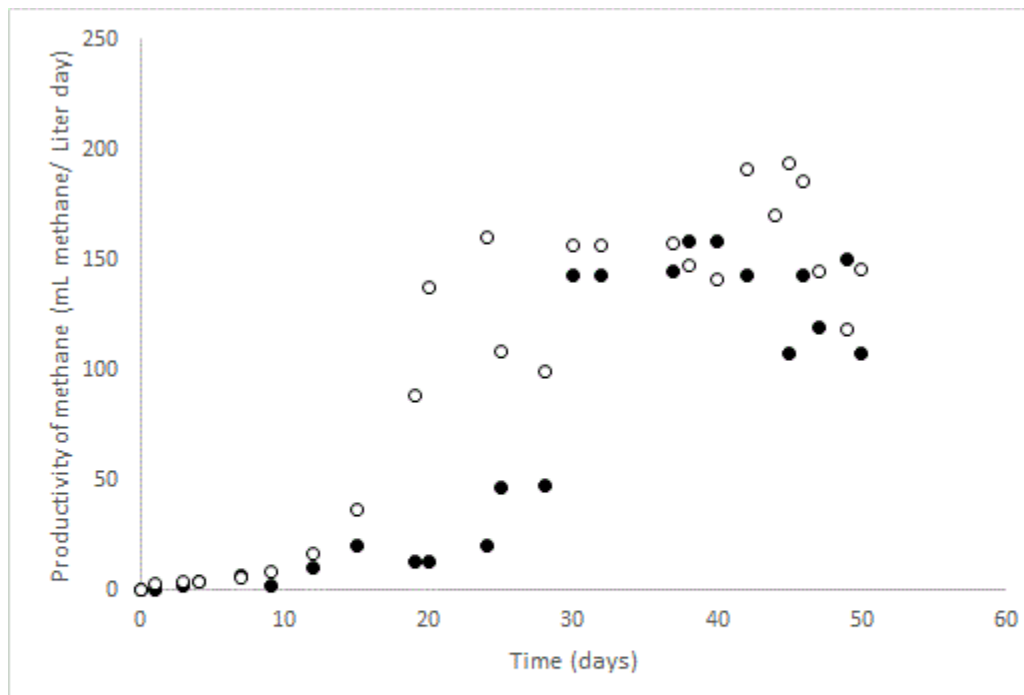


Fig 1. Productivity of methane. The digester 1 is represented for ● and digester 2 is represented for ○

When the digesters reached the steady state the number of haplotypes (DGGE) increased about 40%, while the copies number of functional genes increased twice in



relation to the beginning of the process.

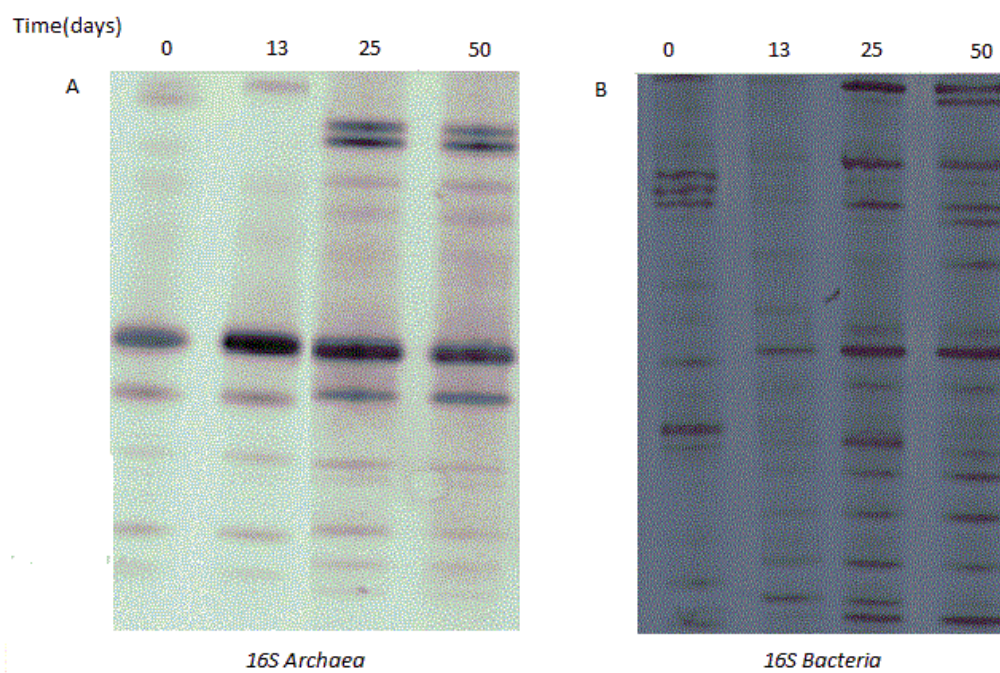


Figure 2 DGGE 16S of digester 1: (A) *Archaea* and (B) *Bacteria*

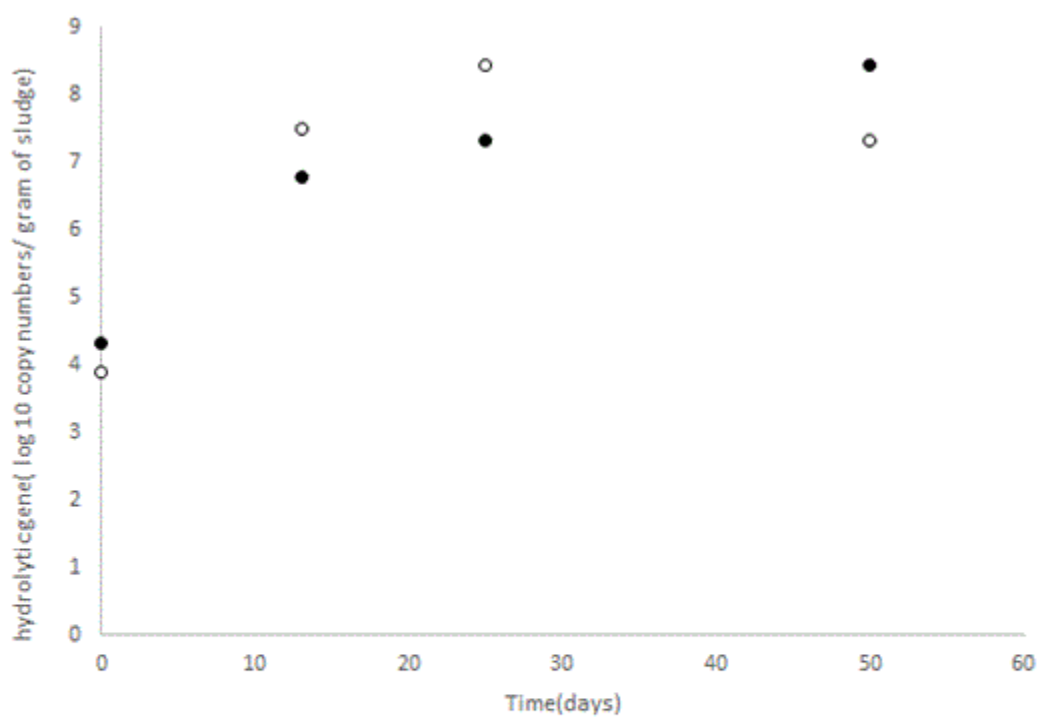


Fig 3. Copy number of hydrolytic gene during start up of two anaerobic digester  
The digester 1 is represented for ● and digester 2 is represented for ○

## MICROBIOLOGICAL SOIL QUALITY IN CITRUS ORCHARD UNDER DIFFERENT MANagements

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### Background

The inter rows of citrus orchards in Brazil are generally managed with mowing, while in the lines mainly use the herbicide glyphosate. There is a great predominance of *Urochloa decumbens* in the inter rows of orchards. As there are reports of allelopathy of *U. decumbens* to citrus and damages that result from the continued use of glyphosate in other cultures.

### Objectives

This project aims to study through microbiological parameters influences on soil quality using two vegetation in inter rows, managed with different mowing, with and without herbicide in the lines of Tahiti acid lime orchard.

### Methods

Since 2010, trial was conducted in Mogi Mirim, São Paulo State, Brazil, in split plot design, where in the plots were composed of two types of vegetation: *U. decumbens* and *U. ruziziensis*; the sub plots for two types of mowing: conventional and ecological; and the sub sub plots: by applying and absence of the herbicide glyphosate on planting line. Samples of soil from the rhizosphere of plants were collected 0-10 cm depth, in three seasons and the following parameters evaluated microbial biomass carbon (BMC), basal respiration (RB) soil and metabolic quotient.

### Conclusions

The results showed that there were significant differences for the microbiological parameters and soil treatment with *U. decumbens*, ecological mowing and herbicide use were better when compared with others. There was no damage to the vegetative and productivity development of Tahiti acid lime, so the changes in management can be recommended.

**FEMS-2539**

**Environmental microbiology - 2**

**EPIBACTERIAL AND PLANKTONIC BACTERIAL COMMUNITIES RESPOND TO INCREASED PCO<sub>2</sub> AND RISING TEMPERATURE LEVELS: A THREE-MONTH MESOCOSM STUDY**

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**Background**

In the framework of the BMBF-funded consortium project BIOACID (*Biological Impacts of Ocean Acidification*) we are interested in the response of marine epibacterial communities attached to biological surfaces to increased *p*CO<sub>2</sub> and temperature. In spring 2014, we performed a three-month experiment in the benthic mesocosm facility at the Alfred Wegener Institute in List (Germany) which allows low and high tide simulation to mimic Wadden Sea conditions. We manipulated the seawater *p*CO<sub>2</sub> and temperature as predicted by the end of this century to simulate ocean acidification and warming events of the future North Sea.

**Objectives**

Both stress factors were tested for single and combined effects on epibacterial communities on the surface of the brown macroalgae *Fucus vesiculosus* forma *mytili* and in comparison on the planktonic microbiota of the surrounding seawater.

**Methods**

Changes in the bacterial phylogenetic diversity were analyzed by bacterial 16S rDNA (V1-V2) amplicon sequencing using the Illumina MiSeq technology.

**Conclusions**

The generated data indicated significant shifts in the bacterial community structure over a period of three months (April to June), driven by rising temperature and lowered seawater pH levels. Overall, our findings suggest significant changes in the microbial diversity under anthropogenic pressures such as global warming and ocean acidification with potentially severe consequences for the future Wadden Sea ecosystem.



**FEMS-0727**

**Environmental microbiology - 2**

**REMOVAL OF LEAD BY PSEUDOMONAS SP ISOLATED FROM KHUZESTAN'S PETROLEUM SOILS, IRAN**

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<sup>2</sup>Microbiology, IAU Kerman Branch, Kerman, Iran

**Background**

Biosorption of heavy metals is effective technology for removal of toxic metals. Algae, bacteria and fungi are useful biosorbents for metal elimination from polluted zones.

**Objectives**

The objective of this study was isolation of lead resistant *Pseudomonas* strains.

**Methods**

Soil samples were homogenized and diluted with sterile normal saline. These samples were cultured in LB agar containing 5ppm of lead in order to isolation of resistant strains. These strains were isolated and cultured in Macconkey agar for isolation of appropriate strains and identified by metabolically tests. Maximum lead resistance of strains was evaluated by MIC test. The bacterial growth were optimized in the presence of lead in various temperatures, rate of shaking and values of pH by spectrophotometry in 600nm at 24h of cultivation. The bacterial strains were tested for ability to grow in the presence of different antibiotic by disc diffusion method. Metal adsorption test measured under optimal conditions by atomic absorption spectroscopy.

**Conclusions**

In this study 24 colonies were resistant to lead and the bacterium, *Msap*, had more resistant than others. The best conditions of growth in temperature, pH and rate of shaking were 37, 9 and 200. The results were verified using the general linear model. The highest of biosorption capacity was 91.79%. The strain, *Msap*, showed resistance to ampicillin, tetracycline, chloramphenicol, erythromycin, and kanamycin. *Msap*, have a great potential for bioremoval of lead from aquatic polluted environments. In conclusion, this study reveals the significance of using the *Pseudomonas* sp in bioremediation of lead contaminated zones.

**FEMS-1874**

**Environmental microbiology - 2**

## **CHARACTERIZATION OF BIOAEROSOLS - VALIDATION OF A REDUCED SAMPLING METHOD -**

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### ***Background***

Bioaerosol monitoring is a rapidly increasing field of environmental risk assessment, which includes measurement of different microbiological and chemical parameters in both indoor and ambient air. Generally, bioaerosols are particles of biological origin with an aerodynamic diameter of up to 100 nm, containing bacteria, fungi, viruses, pollen, plant debris as well as their metabolites like endo-/mycotoxins, allergens and (M)VOC's. In the last years, numerous studies showed associations between biological particles and a wide range of adverse health effects such as infections, intoxication and sensitization.

### ***Objective***

The aim of the study was the validation of a reduced and optimized sampling procedure necessary for a comprehensive bioaerosol risk assessment.

### ***Methods***

We investigated bioaerosols from laying hen-, swine- and cattle livestock on different measuring sites (within the barn, 50 m downwind and background) using impaction and impingement. For the microbiological testing the total viable count of bacteria and fungi as well as a selective enrichment for MRSA, ESBL and VRE was carried out. Additional species identification (bacteria/ fungi) was performed by MALDI-TOF-MS. The chemical characterization included the detection of particulate matter (PM<sub>10</sub>, PM<sub>2.5</sub>, PM<sub>1</sub>, UFP), endotoxin, metal and VOC content.

### ***Conclusion***

The interspecies comparison show clear variation in the microbiological (bacterial/ fungal) and chemical components (PM, endotoxin and (M)VOC level) profile between the livestock (indoor). Up to 50 m downwind no differences compared to the background samples of the corresponding livestock were detected. Due to the harmonized combination of selected chemical and microbiological indicator parameter, the reduced sampling program constitutes a constructive alternative for a comprehensive risk assessment.





**FEMS-0953**

**Environmental microbiology - 2**

## **ISOLATION AND IDENTIFICATION OF CYANIDE TOLERANT BACTERIA FROM WASTEWATER DISCHARGED FROM AN ELECTROPLATING PLANT IN CAPE TOWN, SOUTH AFRICA**

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### **Background**

The pollution of water sources is one of the major challenges municipalities face worldwide. The industrial wastewater discharged into the municipal sewers often contain toxic substances. Electroplating industries contribute to the pollution of water sources with cyanide, cyanide metal complexes and heavy metals used in their metal plating processes.

### **Objectives**

The aim of this study was therefore to isolate and identify cyanide tolerant bacteria from wastewater discharged from an electroplating plant in the Western Cape, South Africa which was suspected of disposing of its potentially hazardous wastewater into the municipal sewers.

### **Methods**

Bacteria were isolated from the biofilm and wastewater discharged from the electroplating plant. The pure colonies were streaked on nutrient agar supplemented with 400 mg/L cyanide to test for tolerance. To characterise and identify the cyanide tolerant isolates the following tests were performed; gram and endospore staining, biochemical tests, selective media, VITEK 2 Compact, Genomic DNA extraction, Polymerase chain reaction (16s rRNA gene amplification) and sequence analysis.

### **Conclusions**

Two of the isolates were identified as *Pseudomonas* species and the other two as *Bacillus* species. The four identification methods used (biochemical testing, selective media, VITEK 2 and sequence analysis (16s rRNA gene) could confirm 100% identity up to the genus level even though a few discrepancies in identifying the species existed. Further studies are however needed to study other genes to differentiate the species. The isolates are further to be tested for cyanide degradation and as possible candidates for bioremediation of cyanide and metal cyanide complexes.



**FEMS-2404**

**Environmental microbiology - 2**

## **MYXOBACTERIA IN PEAT BOGS AND FEN – AN ASTONISHING DIVERSITY**

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### **Background**

Increasingly appearing resistances to antibiotics and the subsequent need for new bioactive substances is one of the fundamental challenges of infection research. Myxobacteria have a rich secondary metabolism, which places them among the best known natural product producers. To date more than 100 new metabolites and more than 500 derivatives were described from these soil dwelling, predatory microorganisms. In the past it turns out that in particular new families, genera and species of myxobacteria are reliable sources for new compounds.

### **Objectives**

Promising sources for the isolation of new myxobacteria are uncommon habitats like, for example, peat bogs or fen, which are characterized by low pH values. This habitat has been neglected with regard to the isolation of myxobacteria in the past. In our study, the diversity of myxobacteria in moor-samples from the Harz-region was evaluated by standard cultivation methods as well as by cultivation independent 16S rRNA-clone bank analyses.

### **Methods**

A total of 297 clones of myxobacterial origin were sequenced and compared to sequences of cultures, isolated from these samples, by phylogenetic analyses. It turned out that the majority of myxobacteria is only represented by clone sequences and could not be cultivated. Comparing these sequences to sequences of a public database (NCBI) revealed that most of these uncultivated myxobacteria are exclusively related to other uncultivated myxobacterial sequences from acidic soils.

### **Conclusions**

This suggests that peat bogs and fen harbor a big diversity of new myxobacterial species, genera and even families which themselves harbor a great potential of urgently needed, new secondary metabolites.

**FEMS-3032**

**Environmental microbiology - 2**

## **EVOLUTIONARY HISTORY INFLUENCES THE SALINITY PREFERENCE OF BACTERIAL TAXA IN WETLAND SOILS**

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### **Background**

Salinity is a major driver of phylogenetic bacterial community composition across the globe and, given the importance of microbial community composition for ecosystem function, it is necessary to understand how salinity structures bacterial communities. If phylogenetic groups exhibit coherence in their salinity preferences, characterizing these preferences will enhance our ability to predict how changes in salinity will affect microbial communities and their associated functions.

### **Objectives**

Consequently, we aimed to examine the response of wetland bacterial community composition and function to changes in salinity and to assess whether salinity preferences were phylogenetically conserved in bacteria.

### **Methods**

To address these aims, we used a reciprocal transplant experiment of fresh- and saltwater wetlands soils.

### **Conclusions**

The salinity of both the origin and host environments affected bacterial community structure (16S rRNA gene sequences, Illumina) and activity (e.g., CO<sub>2</sub> and CH<sub>4</sub> production). Phylotypes were categorized as exhibiting a preference for freshwater, saltwater, or having no salinity preference by comparing presence/absence patterns across treatments. When these preferences were related to phylogeny, a significant influence of evolutionary history was seen in all preference categories. This phylogenetic signature was corroborated by differences in the salinity preferences of high-level taxonomic groups. The majority of  $\alpha$ -proteobacteria and  $\gamma$ -proteobacteria phylotypes preferred saltwater, while the phylotypes of  $\beta$ -proteobacteria were more likely to prefer freshwater, and phylotypes within Verrucomicrobia tended to have no salinity preference. Overall, our results indicate that salinity structures bacterial communities by selecting for organisms with phylogenetically clustered salinity preferences, and these effects on community composition may have consequences for ecosystem function.

**FEMS-0623**

**Environmental microbiology - 2**

**METABOLIC PATHWAY INVOLVED IN H<sub>2</sub> PRODUCTION FROM FORMATE BY  
DESULFOVIBRIO VULGARIS**

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**Background**

Formate is an important metabolite in anaerobic environments, where it is one of the metabolic products of fermentative organisms and an energy source for many bacteria such as sulfate-reducing bacteria (SRB). SRB are anaerobic organisms that possess a high number of formate-dehydrogenases (FDHs) and hydrogenases (Hases), enzymes responsible for H<sub>2</sub> production from formate [1]. In sulfate-limited environments SRB grow fermentatively in syntrophy with other organisms producing H<sub>2</sub>. Moreover, it was recently demonstrated that in the absence of sulfate, *Desulfovibrio vulgaris* displays a very high H<sub>2</sub> production from formate [2].

**Objectives**

In the present work, the electron transfer mechanism involved in formate-driven H<sub>2</sub> production by SRB was studied.

**Methods**

H<sub>2</sub> production by *D. vulgaris* deletion mutants of the two main FDHs ( $\Delta fdhABC_3$  and  $\Delta fdhAB$ ) and of the four periplasmic Hases (Fe-only Hase ( $\Delta hydAB$ ), two [NiFe] Hase ( $\Delta hynAB1$  and  $\Delta hynAB2$ ), and a [NiFeSe] Hase ( $\Delta hysAB$ )) was compared with the wild-type strain in order to disclose the role of each enzyme in the metabolic pathway involved in H<sub>2</sub> production from formate [3].

**Conclusions**

This work contributes to our knowledge about the electron transfer pathways involved in H<sub>2</sub> and formate metabolism in *D. vulgaris*, showing which of the FDHs and Hases play an important role in this process.

[1] Pereira IAC et al (2011) Front Microbiol 2, 69

[2] Martins, M. (2013) Int. J. Hydrogen Energy, 38: 12294

[3] Martins M et al (2014) submitted

**FEMS-1295**

**Environmental microbiology - 2**

**BACTERIAL PROFILING OF SAHARAN DUST DEPOSITION IN THE ATLANTIC OCEAN USING SEDIMENT TRAP MOORINGS – YEAR ONE RESULTS**

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**Background**

Large quantities of dust are transported from the Sahara Desert across the Atlantic Ocean towards the Caribbean each year, with a large portion of it deposited in the ocean. This dust brings an array of minerals, nutrients and organic matter, both living and dead. This input potentially fertilizes phytoplankton growth, with resulting knock-on effects throughout the food chain. The input of terrestrial microbial life may also have an impact on the marine microbial community.

**Objectives**

The aim is to understand the links between dust input and the bacterial community and how this relates to ocean productivity and the carbon cycle.

**Methods**

The current multi-year project consists of a transect of floating dust collectors and sub-surface sediment traps placed at 12°N across the Atlantic Ocean. Sediment traps are located 1200m and 3500m below the sea surface and all are synchronized to collect samples for a period of two weeks.

**Conclusions**

The first set of sediment trap samples were recovered using the *RV Pelagia* in November 2013 with promising results. Results from 7 sediment traps (three at 1200m and four at 3500m) were obtained. In general, the total mass flux decreased as distance from the source increased and the upper traps generally held more material than those at 3500m. Denaturing Gradient Gel Electrophoresis (DGGE) was used as a screening technique, revealing highly varied profiles, with the upper (1200m) traps generally showing more variation throughout the year. Preliminary analysis of 454 sequencing from a subset of the samples supports this observation, and detailed analysis will be presented.

**FEMS-1086**

**Environmental microbiology - 2**

**ISOLATION AND IDENTIFICATION OF BIOAEROSOLS SAMPLED BY PM2.5 FILTERS USED IN NETWORK MONITORING AIR QUALITY ABURRA VALLEY, MEDELLÍN - COLOMBIA**

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**Background**

By the recent years, it has increased the air pollution in urban environments as well as rural, due to anthropogenic activities. This has been reflected on respiratory illness or infections related to air pollutants, mainly to fine inhalable particulate matter PM2.5; nonetheless, it is rarely discussed in the aburra valley about the microorganisms associated to this sort of particles, which can cause human illness ranging from mucosa irritation and allergies to asthma and pneumonia.

**Objectives**

The purpose of this research is the isolation and identification of microorganisms present in PM2.5 filters of the stations MED-UNFM and MED-PJIC of the Air Quality Monitoring Network from the Aburrá Valley

**Methods**

Air sampling equipment BGI PQ200 semiautomatic was used for collection of biological material in PM2.5 Teflon filters 47mm in diameter. A sample of the latter was inoculated and incubated in an enrichment broth; Serial dilutions were made and were inoculated onto selective media. Each of the strains isolated, were characterized morphologically and biochemically (VITEK® 2 Compact).

**Conclusions**

In this research, several microbial species from PM2.5 filters, were isolated and identified like *Bacillus cereus*, *Acinetobacter baumannii*, *Enterococcus faecium* and *Bacillus anthracis*, all those consider as pathological microorganisms. This allows inferring that air quality, besides generating problems associated to respiratory illness, can increase the possibility of contracting bacterial infections.





**FEMS-1240**

**Environmental microbiology - 2**

**METAL-RICH TROPICAL SEDIMENT MICROBIOME UNVEILS A DIVERSE TAXONOMIC AND FUNCTIONAL MICROBIAL COMMUNITY**

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**Background**

The Iron Quadrangle (Minas Gerais, Brazil) is one of the world's largest mining regions, being historically explored for over 300 years. Since then, many toxic metals and metalloids were released into the environment leading to contamination of water bodies. It is well known that microbial community influences the bioavailability of these toxic elements and its importance in the ecological balance of various environments.

**Objectives**

This study investigated the taxonomic and functional diversity of the microbiome from Mina Stream sediment (MSS), historically metal-contaminated, especially arsenic.

**Methods**

The environmental DNA extracted was sequenced using SOLiD and Miseq platforms for the functional and taxonomic analysis, respectively.

**Conclusions**

Taxonomic profile obtained by Greengenes database revealed a complex microbial community, with dominance of *Proteobacteria* and *Parvarcheota*. Contigs recruited bacterial and archaeal genomes, especially *Candidatus Nitrospira defluvii* and *Nitrosopumilus maritimus*, and their presence implicated them in C and N cycling in the MSS. Functional reconstruction revealed a large diverse set of genes for ammonium assimilation and ammonification. These processes have been implicated in the maintenance of N cycle and healthy of the sediment. SEED subsystems functional annotation unveiled a high diversity of metal resistance genes, suggesting that the prokaryotic community is adapted to metal contamination. Furthermore, a high metabolic diversity was detected in MSS, suggesting that the historical arsenic contamination is no longer affecting it. Finally, the results reported herein may contribute to expand the current knowledge of the microbial taxonomic and functional composition of tropical metal-contaminated freshwater sediments.

Supported by: CNPq, CNPq/INCT-Aqua and FAPEMIG

**FEMS-0809**

**Environmental microbiology - 2**

**BIODEGRADATION OF AN ORGANOPHOSPHATE, CHLORPYRIFOS BY MICROBES ISOLATED FROM AGRICULTURE SOIL**

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**Background**

Organophosphorous Pesticides (OP) are being used for pest control in many countries. Extensive use of these compounds results in their accumulation in agricultural soil, water source, affecting insects and humans. Chlorpyrifos (CP) a broad-spectrum insecticide has been used for controlling termites, beetles on paddy fields and vegetable crops. CP acts as a neurotoxin by inhibiting acetylcholinesterase, which degrades the neurotransmission agent, acetylcholine, in the nervous system. Biodegradation of CP results in the formation of a compound TCP (3,5,6-trichloro-2-pyridinol) classified as mobile, toxic also has antimicrobial property.

**Objectives**

Screening, isolation and identification of the microbial isolates for Chlorpyrifos and TCP degradation

**Methods**

Resting cell assay for Chlorpyrifos degradation in liquid media and HPLC analysis

**Conclusions**

Present investigation showed microbial strains isolated and screened from agricultural soil amended with CP. Six bacterial strains (CPD-03, CPD-5C, CPD-7, CPD-18, CPD-20 & CPD-33) showed significant degradation of CP in minimal salt media supplemented with CP @ 10 µg/ml. A resting cell assay was carried out at pH 7.2, 37 °C with CP @ 10 µg/ml and monitored over a period of 48 h, and analyzed using HPLC. The degradation efficiency of these isolates varied between 65% to 67% within 48 h. The isolates CPD-18, CPD-20 and CPD-33 were able to degrade CP and metabolize TCP up to 64% - 65 % within 48 hr compared to CPD-03, CPD-5C, CPD-7 which degraded up to 65% - 67%. These six microbial strains can extend our understanding of pesticide degradation and would be beneficial in using as a consortium for cleaning up pesticide contaminated environment.

**FEMS-1383**

**Environmental microbiology - 2**

**PREVALENCE AND ANTIBIOGRAM PROFILING OF ESCHERICHIA COLI PATHOTYPES RECOVERED FROM TYUME RIVER IN THE EASTERN CAPE PROVINCE OF SOUTH AFRICA**

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**Background**

Waterborne diseases are among the leading causes of morbidity and mortality in developing countries and diarrheagenic *Escherichia coli* are one of the most important etiologic agents of acute diarrhea and represent a major public health problem in developing countries like South Africa.

**Objectives**

In this study, we assessed the prevalence and antibiogram profiles of *E. coli* pathotypes in Tyume River South Africa.

**Methods**

Twelve water samples were collected from three sites along the rivers between August 2010 and July 2011 and *E. coli* was isolated by the membrane filtration method on mFC and Chromocult agar. Identification and pathotyping of the isolates were done by polymerase chain reaction technique using species/pathotype specific primers and antibiogram was conducted using the disk agar diffusion method.

**Conclusions**

Of the 202 presumptive *E. coli* isolates recovered from the river 35% were confirmed as *E. coli*. The pathotypes prevalence was as follows: ETEC (30%); EPEC (35%); EAEC (35%); and EIEC (16%). All five *E. coli* pathotypes showed high-level resistance to ampicillin, tetracycline, cotrimoxazole, and chloramphenicol but were highly susceptible to quinolones, aminoglycosides, and novobiocin. All the EAEC, ETEC, and EIEC pathotypes were resistant against ampicillin, while 87.5% of the EAEC pathotype were resistant to carbenicillin. Also, all the pathotypes were susceptible to quinolones. *TEM* genes and integron conserved segments were observed in 80% of the isolates. The Tyume river appears to be a reservoir of pathogenic *E. coli* strains and antibiotic resistance determinants, and hence a subject of public health importance.



**FEMS-0529**

**Environmental microbiology - 2**

**PHYLOGENETIC CHARACTERIZATION AND COMMUNITY DIVERSITY OF  
HYDROCARBON-DEGRADING BACTERIAL POPULATIONS IN SOIL  
MICROCOSMS ENRICHED WITH VARIOUS AROMATIC HYDROCARBONS**

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**Background**

High quantities of various aromatic hydrocarbons such as toluene, benzene, ethylbenzene and xylene {BTEX} are ubiquitously found in nature as a result of both natural and anthropogenic influences. Despite previous research efforts devoted to understanding the degradation of hydrocarbon pollutants in various environments, yet, there is still paucity of knowledge concerning the diversity of indigenous bacterial populations that are mostly responsible for the biodegradation of various hydrocarbons.

**Objectives**

The primary focus of this study was to isolate and characterize indigenous bacterial populations in soil microcosms previously enriched with various aromatic {BEX} hydrocarbons. Also, to compare the diversity of hydrocarbon degraders between the three microcosm communities examined.

**Methods**

Combinations of standard microbiological {phenotypic} and molecular {16S ribosomal RNA gene sequencing} were used to isolate, characterize and identify 45 bacterial isolates with putative hydrocarbon-degrading capability in the three soil microcosms. Additionally, various bioinformatics and analytical tools were employed for sequence alignments and diversity measurements.

**Conclusions**

A total of 45 bacterial isolates belonging to 5 distinct phyla were phylogenetically characterized among indigenous bacterial populations with putative hydrocarbon-degrading in the soil microcosms. In general, bacterial members of the  $\gamma$ -proteobacteria {mostly *Pseudomonas* and *Acinetobacter*} dominated {between 60 to 94%} among the isolates from the three microcosms. Bacterial members belonging to the  $\beta$ -proteobacteria {*Comamonas* and *Delftia* sp} and Firmicutes {*Bacillus* sp} were also represented. Overall, the differences observed in bacterial phylotypes among the microcosms are probably attributable to the direct effects of the chemical properties of each hydrocarbon pollutant on the indigenous microbial community.

**FEMS-2283**

**Environmental microbiology - 2**

## **NOSOCOMIAL INFECTIONS IN NIGERIA: A CASE STUDY FROM DELTA STATE, NIGERIA**

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### **Background**

Nosocomial or hospital acquired infections (HAIs) is a serious global public health issue, causing the suffering of about 1.4 million people across the world at any given time

### **Objectives**

This study was undertaken to ascertain the prevalence of HAI in Delta State of Nigeria.

### **Methods**

This is a descriptive case study design in which structured questionnaire was administered to 96 doctors, 170 nurses, 24 pharmacists and 40 medical laboratory scientists from randomly selected government hospitals and 90 doctors, 180 nurses, 30 pharmacists and 30 medical laboratory scientists from randomly selected private hospitals, all located in the 25 Local Government Areas of Delta State. The study was carried out between May and July 2013 after Ethical Approval was granted by the Ministry of Health, Delta State.

The commonest HAIs encountered are Urinary Tract Infection (61.4%) followed by Hospital acquired Pneumonia (55.6%) and Staphylococcus aureus (54.4%) for the government hospitals. In the private sector, Hospital acquired Pneumonia (66.1%) is the commonest. The study also showed that the frequency of occurrence of HAI is higher in government than private hospitals. This can be attributed to the higher population of patients leading to overcrowding. The modes of transmission of hospital acquired pneumonia was observed to follow this order airborne > contact with blood and body fluids > contaminated instruments > contaminated hands > needle sticks.

### **Conclusions**

The prevalence of nosocomial infection in this state is very high. Efforts are needed to reduce it, improve the quality of life of patients and healthcare workers and improve healthcare delivery.

**FEMS-1055**

**Environmental microbiology - 2**

**BACTERIAL BIODIVERSITY IN THE RICE RHIZOSPHERE AND HIGH-SALT SOIL AND SEDIMENT ENVIRONMENTS FROM THE CAMARGUE REGION (FRANCE)**

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**Background**

The Camargue territory in the south of France is a delta triangle of 1500 km<sup>2</sup> of the Rhône River as it flows into the Mediterranean. This zone consists mostly of salty land inhabited by a diverse community of birds, wild white horses, black bulls and waterfowl, the most famous of which are pink flamingos. Rice production covers approximately 10,000 hectares which accounts for 30 percent of France's rice consumption.

**Objectives**

We are examining the microbial composition of high salinity salt ponds, soils and the rhizosphere of rice plants in the Camargue region for two consecutive years (2013-2014) in order to establish a variable-temporal comparison regarding the physical and chemical properties of the environment versus the bacterial communities present in similar (saline) environments.

**Methods**

Bacterial biodiversity of five rhizosphere sites, growing two varieties of rice (Arelate and Gageron), plus sixteen sites of sediments and soils from nearly saline wetlands, were studied using pyrosequencing of PCR-amplified 16S V3-V4 rDNA amplicons from total extracted genomic DNA.

**Conclusions**

Our preliminary results revealed bacterial populations dominated by members of the *Bacteroidetes* and *Proteobacteria* phyla. However, in sandy soils with a lower degree of salinity than the 25 ppm, we observed, members from the *Acidobacteria* phylum representing an important fraction. Ongoing analyses of diversity (Chao1, Shannon and Simpson index) and statistics such as principal component analyses (PCA) will permit a detailed description of the bacterial populations among the different sampling sites, and better comparisons with similar types of sites worldwide.





**FEMS-1556**

**Environmental microbiology - 2**

## **INVESTIGATION OF AEROBIC HETEROTROPHIC BACTERIA IN OIL RESERVOIRS IN THE SOUTHEAST ANATOLIA REGION**

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### **Background**

Petroliferous soil has unique environment due to the presence of a variety of aliphatic and aromatic hydrocarbons, creating a distinctive habitat for certain microbial communities. Although hydrocarbons are constituted only of two elements, relative to their electron acceptors; they are exploited as growth substrates by numerous microorganisms. Some aerobic bacteria, especially *Pseudomonads* are one of the most frequently isolated bacteria from hydrocarbon-impacted environments due to their reported metabolic pathways.

### **Objectives**

The aim of this study was to determine the presence, abundance and the predominant culturable aerobic heterotrophic bacteria as well as *Pseudomonads* in 4 different production wells located in the Southeast Anatolia Region.

### **Methods**

Petroleum samples were warmed to 40-60°C for a few hours until water-phase could be observed under oil phase. Diluted water-phase samples were inoculated onto Nutrient Agar, Tryptone Soy Agar, R2A, Cetrimid Agar and Pseudomonas Agar Base with and without salt. Petri plates were incubated at 30°C for 2 days (R2A for 5 days). Genomic DNA was extracted from the pure cultures using UltraClean Microbial DNA Isolation Kit. 16S rDNA gene fragments were amplified using U968F and L1401R primers. The amplified gene fragments were subsequently analyzed using sequencing.

### **Conclusions**

It was determined that the number of aerobic heterotrophic bacteria and *Pseudomonads* was generally low/high (min. 9 cells/ml and max. 1358 cells/ml) in the oil reservoirs. 15 pure cultures were obtained from the samples. Among the cultured bacteria, *Acinetobacter guillouiae* (99.3%), *Halomonas daqingensis* (99.7%), *Suttonella indologenes* (93.1%) and *Pseudomonas stutzeri* (99.8%) were present.

**FEMS-2901**

**Environmental microbiology - 2**

**EVALUATING THE RELATIVE INFLUENCE OF BIOTIC AND ABIOTIC FACTORS  
ON EFFICIENCY OF BIOAUGMENTATION MEDIATED EX-SITU  
BIOREMEDIATION**

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**Background**

Soil and ground water pollution with xenobiotic chemicals is a major environmental concern. Bioremediation has been regarded as an efficient and environment friendly approach for restoration of contaminated niches. Amongst different bioremediation procedures, bioaugmentation is considered as one of the most potent approach for bioremediation. However, till date very few reports have shown successful application of this approach. It is often suggested that variety of biotic and abiotic factors e.g. indigenous microbial community, physico-chemical nature of contaminating pollutant and target soil may influence the efficiency and outcome of bioaugmentation process.

**Objectives**

To systematically address this issue, we performed a study for determination of relative influence of biotic and abiotic factors on efficiency of *p*-nitrophenol (PNP) degradation during bioaugmentation mediated *ex situ* bioremediation.

**Methods**

PNP degradation was monitored in microcosms prepared with soil samples collected from seven geographically distinct Indian provinces (viz., Assam, Andhra Pradesh, Gujarat, Karnataka, Maharashtra, Rajasthan and Tamil Nadu) having diverse physico-chemical character and native microbial communities. Soil samples were spiked with 70 ppm of PNP and subjected to bioaugmentation using pre-grown cells of degradative strain.

**Conclusions**

Quantification of residual PNP showed apparent enhancement of pollutant degradation; however, kinetics of pollutant removal varied significantly as a function of soils properties. Results from statistical analysis of PNP degradation patterns and matrices generated with microbial community and physico- chemical characteristic of soil highlighted relatively strong influence of indigenous bacterial community on biodegradation efficiency. It was observed that soil microbial community renders such impact by differentially modulating the survival of bioaugmented strain.

**FEMS-3016**

**Environmental microbiology - 2**

**AN O-NITROBENZOATE (ONB) SENSITIVE TRANSPOSON MUTANT OF ARTHROBACTER PROTOPHORMIAE STRAIN RKJ100 INDICATES FOR INVOLVEMENT OF N-ACETYLGLUCOSAMINIDASE IN BACTERIAL RESISTANCE TOWARDS ITS ELEVATED CONCENTRATIONS**

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**Background**

Microorganisms capable of thriving under extreme environments are of great significance for basic as well as applied studies. Consequently, microorganisms showing survival under extreme environments have attracted considerable interest. Several studies have shown isolation and characterization of extremophilic microorganism. However, relatively fewer studies have reported isolation and characterization of microorganisms from niches characterized by elevated concentration of anthropogenic, xenobiotic environment pollutants. Previously we had characterized *Arthrobacter protophormiae* RKJ100 for its ability to tolerate extremely high concentrations of o-nitrobenzoate (ONB), a toxic xenobiotic environmental pollutant.

**Objectives**

Main objective of present study was to characterization of gene(s)/ gene cluster(s) involved in resistance of strain RKJ100 towards elevated concentrations of ONB.

**Methods**

Random transposon mutants of strain RKJ100 were generated and screened for ONB sensitive phenotype. A sensitive mutant was defined as one which exhibits sensitivity towards ONB at  $\geq 30$  mM. Molecular and biochemical characterization of this mutant was carried out to identify the gene disrupted due to the transposition.

**Conclusions**

Results from the characterization of ONB sensitive mutants showed disruption of endo- $\beta$ -N-acetylglucosaminidase (ENGase) gene. ENGase is a non-essential enzyme involved in oligosaccharide processing and cell wall recycling many life forms. It has been shown to have role in cellular homeostasis. Results obtained

during this study present first evidence for its role in bacterial resistance towards elevated concentrations of toxic xenobiotic compound.

**FEMS-2720**

**Environmental microbiology - 2**

**BIODEGRADATION OF BISPHENOL A-LIKE ENDOCRINE DISRUPTORS BY DIVERSE MICROORGANISMS FROM RIVER SEDIMENT**

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**Background**

Bisphenol A (BPA) is a widely used chemical in the production of plastics and resins. Tetrabromo-BPA (TBBPA), the BPA derivatives with bromide atoms, is one of the most commonly used brominated flame retardants. The broad distribution of BPA-like pollutants in the environment raises great concern because of their endocrine-disrupting characteristics and toxicity.

**Objectives**

Developing cheap and low environmental impact remediation methods for BPA-like pollutants is emergent. Therefore, the biodegradation abilities of diverse microorganisms toward BPA and TBBPA were investigated.

**Methods**

The microcosm from acclimated sediment culture was used to evaluate the biodegradation ability of diverse microorganisms and isolate pure degraders.

**Conclusions**

The microcosm completely removed BPA (10 mg L<sup>-1</sup>) within 28 h and transformed the contaminant into several metabolic intermediates. During the degradation process, the microbial composition fluctuated and finally restricted to *Pseudomonas knackmussii* and *Methylobacterium clara*. The four isolated BPA degraders were all genetically similar to *P. knackmussii*. Although the degradation ability of mixed strains was higher than that of single strain, it was far less than that of the original microcosm. These results demonstrated the novel role of *P. knackmussii* in BPA degradation, as well as the crucial role for microbial diversity in pollutant decomposition. From the acclimated sediment culture, a novel TBBPA degrader was isolated. It degraded 70 % of the pollutant (4 mg L<sup>-1</sup>) within 5 days and transformed the pollutant to one degrading by-product. Our results are helpful to understand the natural attenuation processes of BPA-like endocrine disruptors, as well as to remediate those structural similar pollutants.

**FEMS-2639**

**Environmental microbiology - 2**

## **PRESENCE OF *P. SHIGELLOIDES* IN TEMPERATE CLIMATE ZONE LAKES**

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### **Background**

*Plesiomonas shigelloides* is a Gram-negative rod-shaped bacterium found in aquatic environments. Most of the reports on its isolation are from tropical or subtropical areas. Furthermore, the bacterium is considered as an emerging opportunistic pathogen.

### **Objectives**

Aim of this study was to detect presence of *P. shigelloides* in lakes situated in temperate climate zone (Central Europe).

### **Methods**

Total of 42 water samples taken from Vojvodina, i.e. northern Serbia and southern Hungary lakes were examined. Some samples were taken from same locations but at different time intervals, so all isolates were obtained from geographically and/or chronologically different sources. The samples were spread directly onto Inositol Brilliant Green Bile Agar and incubated at 37 °C for 24 hours. The colonies of inositol fermenting bacteria were randomly selected, subcultivated and several preliminary tests were performed (Gram staining, catalase and oxidase test, indol production, gelatin hydrolysis and citrate utilization test). *P. shigelloides* was additionally characterized and identification was confirmed by PCR using specific primers (Ref, god). Additionally, RAPD analysis was performed to estimate genetic relationships among the isolates using 5 different primers.

### **Conclusions**

The results indicate the frequent presence of bacteria in the waters in moderate climate, since *P. shigelloides* was isolated from 81% of the samples. RAPD analysis revealed distinct DNA fingerprint patterns for each *P. shigelloides* strain. The results confirm high presence of *P. shigelloides* in temperate climate zone lakes in Central Europe, which is probably a result of global climate changes. In addition, *P. shigelloides* can represent a potential health concern in this region.

**FEMS-2905**

**Environmental microbiology - 2**

## **THE SOIL MICROBIOME: INFLUENCE OF THE DECADES-LASTING INDUSTRIAL PRODUCTION OF BETA-LACTAM ANTIBIOTICS**

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### **Background**

Factory sites, wastewater treatment plants or hospitals are representative environments containing high concentrations of xenobiotics.  $\beta$ -lactam antibiotics (BLA) in these environments could directly exert a selective pressure on bacteria, resulting in spontaneous mutations or horizontal transfer of genetic elements among them. These genetic changes could pose a threat to human health, if these bacteria become multiresistant pathogens and migrate to the clinical settings. Our knowledge about the taxonomic composition of bacterial consortia from BLA contaminated environments is scarce.

### **Objectives**

To investigate and characterize bacterial community subjected to long-term selection pressure of BLA, soil samples from the factory site of a pharmaceutical company, which has been producing  $\beta$ -lactam antibiotics for more than 50 years, were taken, analyzed, and taxonomic compositions were compared to those obtained from non-polluted soils of the same area.

### **Methods**

In this study we assessed the microbial diversity on three different sites among the factory campus and two control sites (approx. 5km from the factory) using tag-encoded 454pyrosequencing of the 16s rRNA genes.

### **Conclusions**

The selection pressure of BLA did not reduce the diversities of bacterial consortia compared to those in the reference samples, although composition of the dominant species was different. The data from culture dependent study showed that the overall resistome of the polluted soil communities was boosted not only in terms of

resistance to BLAs but also within the tetracycline, macrolide, chloramphenicol, or aminocyclitol class of antibiotics.

The data demonstrate a very strong selective pressure imposed by anthropogenic activities on the transfer of resistance determinants among bacteria.



**FEMS-0820**

**Environmental microbiology - 2**

**PLACKETT-BURMAN STATISTICAL DESIGN TO IMPROVE THE CULTURE MEDIA COMPOSITION AND THE ENZYME ACTIVITY OF TWO RECOMBINANT LACCASES IN PICHIA PASTORIS**

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**Background**

Laccases are multicopper oxidases widely distributed in nature and catalyze the transformation of aromatic and non-aromatic compounds with reduction of molecular oxygen to water. We cloned the syntetic optimized genes, *GILCC1* and *POXA 1B* from *Ganoderma lucidum* and *Pleurotus ostreatus* respectively in *Pichia pastoris*. In expression experiments we obtained enzymatic activities of  $451.08 \pm 6.46 \text{ UL}^{-1}$  and  $0.13 \pm 0.028 \text{ UL}^{-1}$  for *GILCC1* and *POXA 1B* respectively. We are planing to employ recombinant laccases for the degradation process of kraft pulping or dye removal from effluents.

**Objectives**

Objective was to improved the enzymatic activity of each cloned genes by improving the culture media composition.

**Methods**

We use a Plackett-Burman statistical design to evaluate media volume, inocula percentage, copper, glucose,  $\text{NH}_4\text{SO}_4$ , peptone and yeast extract concentration, each one of them with two levels, to detect the positive or negative influence and contribution percentage of each one.

## Conclusions

In both cases we improved the enzymatic activity by optimizing culture media composition. In the case of *GLCC1*; treatments 1 and 9 surpassed  $1.5 \text{ UL}^{-1}$ ; which exceeds the maximum activity obtained in previous trials  $0.13 \text{ UL}^{-1}$ ; being treatment 1 which achieved higher laccase activity  $4.69 \text{ UL}^{-1}$  meaning an increase of 36.07 times. In the case of *POXA 1B*; treatments 4 and 11 surpassed  $1200 \text{ UL}^{-1}$ ; exceeding the maximum activity obtained in previous trials  $451.08 \text{ UL}^{-1}$ ; being treatment 11 which achieved higher laccase activity  $1373.72 \text{ UL}^{-1}$  meaning a 3-fold increase enzyme activity. For both laccases genes the culture time of *P. Pastoris* was 168 hours.

**FEMS-0821**

**Environmental microbiology - 2**

**SEQUENTIAL STATISTICAL DESIGNS FOR IMPROVEMENT A RECOMBINANT LACCASE PRODUCTION IN PICHIA PASTORIS IMMOBILIZED CELLS**

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**Background**

We cloned the syntetic optimized gene, *POXA 1B* from *Pleurotus ostreatus* in *Pichia pastoris*. In expression experiments we obtained an enzymatic activities of  $451.08 \pm 6.46 \text{ UL}^{-1}$ . We are planing to employ a packed bed column, with the recombinant immobilized cells for dye removal from effluents.

**Objectives**

Objective was to improved the enzymatic activity of recombinant *POXA 1B* by improving the culture media composition in a bath culture of immobilized cells of the recombinant strain.

**Methods**

We use a Plackett-Burman statistical design to evaluate media volume, copper,

glucose,  $\text{NH}_4\text{SO}_4$ , peptone and yeast extract concentration, each one of them with two levels, to detect the positive or negative influence and contribution percentage of each one. After that, a Box-Behnken design allow us to optimize the more influent factor by analyzing the three levels of factor interaction through a response surface.

### **Conclusions**

We improved the enzymatic activity from 14.4U/L at 156h (first immobilized cell assay) to 1300U/L at 168 h of immobilized cells bath culture (after statistical optimization), meaning a 90.3-fold increase enzyme activity at 168 hours. The recombinant activity of the recombinant enzyme *POXA 1B* produced by using immobilized cells; exceed the maximum activity obtained in previous free cells trials 451.08  $\text{UL}^{-1}$ ; meaning a 2.88-fold increase enzyme activity at 168 hours.

**FEMS-0668**

**Environmental microbiology - 2**

**MICROBIOLOGICAL STUDY OF SEWAGE SLUDGE COMPOSTING USING MICRO-POROUS LAMINATE MEMBRANE COVER SYSTEM TECHNOLOGY**

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**Background**

Treatment of wastewater involves generation of large amounts of solid wastes worldwide, known as sewage sludge. Composting of sewage sludge from wastewater treatment plants (WWTPs) is considered a successful strategy for its sustainable recycling.

**Objectives**

The aim of this study was to investigate the bacterial community structure and population dynamics during composting, using both cultivation-dependent and cultivation-independent techniques, to gain knowledge contributing to the improvement of the efficiency of the process.

**Methods**

The composting process was conducted during 180 days in an industrial composting plant. Samples were retrieved from a full-scale pile with semi-permeable Gore-tex covers, in combination with an air insufflation system. Waste sludge from an urban WWTP was mixed with vegetal pruning wastes as bulking agent. Microbial characterization included: enumeration of viable (platable) cells, measurements of enzyme activities, phylogenetic identification of isolates by 16S-RNA gene sequencing, and characterization of bacterial communities by a next-generation, Illumina-based sequencing approach.

**Conclusions**

Composting of sewage sludge under microporous-laminate membrane covers enhanced the degradation process, compared to other composting technologies. Changes in enumeration of cultivable microbiota took place during the 15 first days of composting. High activities of enzymes dehydrogenase, protease and arylsulphatase were observed during the first five days, suggesting a high rate of substrate degradation. Pyrosequencing data demonstrated that bacterial diversity was strongly influenced by the stage of the process. The greatest bacterial diversity was observed between days 15 and 30. These results suggest that composting under microporous-membrane covers can improve the process performance and reduce the operating cost.

**FEMS-3035**

**Environmental microbiology - 2**

## **COMMUNITY COMPOSITION AND DIVERSITY OF N FIXING CYANOBACTERIA ASSOCIATED WITH MOSSES IN SUB-ARCTIC ECOSYSTEMS**

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### **Background**

Moss associated cyanobacterial communities (MAC) are thought to be the major drivers of the Nitrogen (N) budget at high latitudes. Most studies have been carried out in the boreal forest and the High Arctic, whereas biological N fixation in other moss-rich regions e.g. sub-arctic alpine ecosystems may also be largely MAC-based.

### **Objectives**

To evaluate diversity, abundance and N fixation activity of cyanobacteria associated with the four moss species *Racomitrium lanuginosum*, *Hylocomium splendens*, *Pleurozium schreberi* and *Sanionia uncinata*, all abundant in Icelandic ecosystems.

### **Methods**

Moss samples were collected from three moss-dominated ecosystems in Iceland. Cyanobacterial identification and quantification was carried out by phase-contrast, fluorescence and confocal scanning microscopy. Estimation of cyanobacteria relative abundance was performed by amplification and sequencing of *nifH* genes. N fixation was assessed with the acetylene reduction assay (ARA).

### **Conclusions**

The cyanobacterial strains identified appeared to be from the orders *Stigonematales* and *Nostocales*. N fixation varied over time, also it was responsive to microclimatic/micro-topographic gradients. Simulated climate warming and grazing negatively affected N fixation activity. Moss water content and type of vegetation were the most influent parameters on potential N<sub>2</sub> fixation activity. Our finding about MAC in moss-widespread sub-arctic ecosystems may have substantial impact on the understanding of the N cycle in this terrestrial environment.



## CHARACTERIZATION OF BACTERIAL COMMUNITY ASSOCIATED WITH THE DEGRADATION OF PIG CARCASSES

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### Background

Foot-and-mouth disease virus causes a highly contagious vesicular disease of domesticated and wild ruminants and pigs. A significant volume of infected livestock carcasses should be disposed of safely, economically and environmental-friendly.

### Objectives

Composting is one of strategies for the safe disposal of infected livestock carcasses. In this study, the bacterial community associated with the degradation of pig carcasses was characterized in the composting process of them.

### Methods

The compost bin was constructed with concrete frames for the walls and floor. Two-layer stainless steel grids were installed to put the carcasses of 4 pigs (80~90 kg/individual) in the bin. The bin was filled with well-mature compost mixture. To collect compost sample, perforated stainless steel tubes, which have been specially designed to collect sample without disturbance, were installed in the bin. Bacterial community was analyzed using 16S rDNA-PCR and pyrosequencing.

### Conclusions

The results of volatile organic compounds (VOCs) monitoring indicated that the easier degradable fractions of pig carcasses decomposed during initial 50 days, and most of pig carcasses decayed during 170 days. The VOC concentrations at the top layer of compost bin were lower than VOCs at the bottom, indicating the top layer of compost can play a role as biofilter for the removal of malodor gases. Dominant bacteria at the initial stage were *Alcanivorax*, *Aquimarina*, *Atopostipes*, *Bacillus*, *Cerasibacillus*, *Pseudomonas*, *Psychrobacter*, *Terribacillus* and *Ulvibacter*. At the active degrading period of pig carcasses, *Alcanivorax*, *Bacillus*, *Clostridium*, *Ignatzschineria*, *Lentibacillus*, *Pelotomaculum*, *Sporanaerobacter* and *Tissierella* were dominated. *Caldicoprobacter*, *Clostridium*, *Sedimentibacter*, *Syntrophaceticus* and *Virgibacillus* were followed during final composting period.



**FEMS-2677**

**Environmental microbiology - 2**

## **POTENTIAL USE OF YEASTS ISOLATED FROM FORESTED WETLAND IN AZO DYES DECOLORIZATION**

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### **Background**

Forested wetlands are interfaces between terrestrial and freshwater systems and play an important role in the hydrology and nutrient filtration, acting as carbon sinks and in biodiversity regulation and maintenance. Also, despite microbial biodiversity in these systems is high, the biotechnological potential of this microbial diversity has been almost ignored, particularly yeast communities.

### **Objectives**

The main purpose of this study is to evaluate the potential exhibited by yeast isolates in decolorizing different azo dyes, for future use in bioremediation.

### **Methods**

In this work, wetlands have been screened for wild yeast isolates exhibiting ability to degrade five different azo dyes, a major group of synthetic dyes highly recalcitrant to current degradation processes. From the universe of isolates selected based on different colonial morphologies, 92 yeast isolates were randomly chosen for assessing their decolorizing abilities. Hierarchical clustering, based on medium color changes during incubations, were used to evaluate differences in the performance of azo dyes decolorization.

### **Conclusions**

Yeast isolates differed markedly in their ability to decolorize the tested dyes. Among the best eleven isolates selected and identified to the species level by molecular methods, the basidiomycetous yeasts *Cryptococcus laurentii* AGG729 and *Cryptococcus podzolicus* AGG691, showed the highest dyes removal capacity. Our

results pointed out that yeasts occurring in forested wetlands, have potential to be used in biotechnological processes, being azo dyes bioremediation a case in point.

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FEMS-1910

Environmental microbiology - 2

## CHARACTERIZATION OF MICROBIAL COMMUNITIES WITHIN PETROLEUM REFINERY WASTES AND THEIR CATABOLIC POTENTIAL IN BIOREMEDIATION

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### Background

Autochthonous microbial communities within the highly hazardous petroleum contaminated sites could play significant role in *in situ* bioremediation. Unlike oil spill and other hydrocarbon contaminated environments, microbial diversity and their remedial potential is least studied within petroleum refinery wastes.

### Objectives

During the present study microbial diversity and their catabolic potential relevant for *in situ* bioremediation of petroleum refinery waste were studied.

### Methods

Gas chromatography mass spectrometry, ICP, and other standard methods were used to characterize the waste sludge. Orion multiparameter meter was used to determine DO, pH, ORP, temperature on site. 16S rRNA gene sequences were obtained from metagenomes through Illumina (GAIIx) sequencer. Clone library based analysis was done to reconfirm the archaeobacterial populations. 65 bacterial strains were isolated through plating on R2A medium. BIOLOG eco plates were used to ascertain community level physiological profiles.

### Conclusions

Dominance of anaerobic, moderately thermophilic, fermentative, sulphate-, iron-, nitrate- reducing, bacterial genera like *Fervidobacterium*, *Coprothermobacter*, *Thermodesulfovibrio*, *Deferribacter*, *Nitrospira*, *Anaerolinea*, *Thermoanaerobacterium*, etc. was observed. Presence of methanotrophic and methanogenic, acetoclastic, hydrogenotrophic, archaea (*Methanoseata*, *Methanolinea*, *Methanocella*, etc.) was confirmed by both NGS and clone library analysis. Bacterial strains isolated from the samples showed their affiliation to genera *Pseudomonas*, *Bacillus*, *Burkholderia*, *Enterobacter*, *Kocuria*, *Microvirgula*, *Pandoreae* etc. The isolates were screened for their ability to grow in presence of various hydrocarbons (Naphthalene, Dodecane, etc.) crude oil, oily sludge, biosurfactant production, varied temperature (15°C - 45°C), pH (3.0 - 9.0) and heavy metals. The overall study illustrated microbial community structure and their potential in survival and hydrocarbon biotransformation, which may be exploited for *in situ* bioremediation strategies.

**FEMS-2935**

**Environmental microbiology - 2**

**CHARACTERIZATION OF THE FUNGAL ICE NUCLEATION MACHINERY BY MEANS OF  
FUSARIUM ACUMINATUM**

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**Background**

Freezing of water above -36°C is based on ice nucleation activity (INA) mediated by ice nucleators (IN), and biological particles are a potentially important source of IN in the atmosphere. INA of the fungal genus *Fusarium* was already described about 30 years ago and INA of *Fusarium* as well as INA of other fungal genera is assumed to be mediated by proteins or at least to contain a proteinaceous compound. However, to date the precise INA machinery of *Fusarium* remains unidentified.

**Objectives**

In this study we use analytical techniques to identify INA protein in surface protein extract from *Fusarium acuminatum*. The ultimate Goal is to elucidate the biological and environmental impact of fungal IN.

**Methods**

We use liquid chromatography, customized freezing assay, SDS-page, mass spectrometry and molecular methods to subsequently analyze the fungal IN machinery.

**Conclusions**

Preliminary results show that the INA protein of *F. acuminatum* is contained in the early size exclusion chromatography fractions indicating a high molecular size. Moreover we could identify a single protein band from IN active fractions at 130-145 kDa corresponding to sizes of IN proteins from bacterial species. To our knowledge this is for the first time an isolation of a single protein from *in vivo* samples, which can be assigned as IN active from *Fusarium*.

**FEMS-1639**

**Environmental microbiology - 2**

## **POPULATION ANALYSIS OF BACTERIA IN A LOW TEMPERATURE WATER CATCHMENT SYSTEM**

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### **Background**

Drinking water supplies are based on groundwater resources all over the world. At some locations problems with higher concentrations of iron and manganese in groundwater can occur. The principle of subsurface iron and manganese removal are periodically injections of aerated water into an anoxic aquifer through a well, resulting in the precipitation of iron and manganese hydroxides. However, the precipitation of insoluble oxyhydroxides caused by chemical and microbial processes may result in the clogging of the aquifer and pumps.

### **Objectives**

Bacterial communities of water wells from a water catchment system with subsurface iron and manganese removal were determined to identify key bacteria. Phylogenetic diversity and relative abundance of bacteria in water samples were compared between wells already in operation to those not yet operated.

### **Methods**

To estimate well-clogging, qPCR with universal primers was performed for all samples and specific primers targeting (i) *Rhodoferax* spp., (ii) *Crenothrix polyspora* and (iii) *Gallionella* spp. were applied. Furthermore, PCR-DGGE technique and 454-pyrosequencing were used to observe bacterial alterations within water samples.

### **Conclusions**

Our results showed that wells in operation contained a higher alpha-diversity, and bacterial communities were dominated by *Rhodoferax* and *Methylobacter*. Abundant groups in not yet operated wells were *Gallionella* spp. and *Crenothrix polyspora*. Redundancy analyses were performed to determine a relationship between samples and environmental factors. The parameters nitrate, iron(III) oxide, iron(II) oxide and ammonia create differences between bacterial communities in operated and not yet operated wells.

The diversity and composition of native microbial populations in water samples differed fundamentally from those influenced by operation.



**FEMS-0691**

**Environmental microbiology - 2**

## **MARINE HYDROCARBONOCLASTICUS BACTERIA AS WHOLE-CELL BIOSENSORS FOR N-ALKANES**

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### **Background**

Whole-cell biosensors are useful systems for the *in-situ* monitoring of seawater for hydrocarbons derived from oil spills. Currently available biosensors for hydrocarbons show limitations derived from the low solubility in water of hydrocarbons or the high ionic strength of seawater.

### **Objectives**

We compared the performance of a biosensor system for the detection of short chain alkanes in seawater, hosted in either *E. coli* (commonly used in many whole-cell biosensors but not optimized for alkane degradation) or different marine hydrocarbonoclastic bacteria specialized in assimilating alkanes.

### **Methods**

A set of reporter strains containing a plasmid harbouring the reporter circuit was constructed. Fluorescence assays were carried out to study the time-dependent response of the reporter strains to different alkanes at different concentrations, and the response of reporter strains to real samples such as seawater contaminated with petrol or crude oil.

### **Conclusions**

Some of the marine hydrocarbonoclastic bacteria tested performed better than *E. coli*. These strains showed very good response to seawater containing pure alkanes, petrol and crude oil in just 2 hours. *Alcanivorax borkumensis* proved to be the best host for the biosensor system.

**FEMS-0740**

**Environmental microbiology - 2**

## **HOW THE PRESENCE OF ORGANIC POLLUTANTS AND METALLOIDS CAN INFLUENCE DIBENZOTHIOPHENE -DEGRADING BACTERIAL STRAINS**

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### **Background**

In an oil polluted site, several components of environmental concern can be found including Polycyclic Aromatic Hydrocarbons (PAH), Dibenzothiophene (DBT) and metals. Studies showed that the naturally occurring soil bacteria are able to transform them to safe end products (bioremediation).

### **Objectives**

1- To find tolerance of *Burkholderia fungorum* DBT1 (B.DBT1) and *Burkholderia fungorum* 95 (B.95) to DBT and PAH mixture and in planktonic and biofilm forms 2- Evaluating the growth and transformation ability of these strains in presence of selenium and tellurium as sample inorganic pollutants.

### **Methods**

The growth as biofilm in 96 well plate was established for both strains using Calgary Biofilm Device (CBD) with the method described by Ceri et al., 1999. Growth curves were drawn based on CFU counting on YMA plates. Biofilm photos were taken by Confocal Microscopy. Selenite and tellurite reduction efficacy assessment was performed based on protocols described by Kessi et al, 1999 and Turner et al, 1992 respectively.

### **Conclusions**

Both strains can tolerate high concentrations of DBT (2048 ppm) in both planktonic and biofilm forms while the mixture of PAH can only be tolerated by planktonic form for both strains. B.95 is able to tolerate the maximum used concentration of selenite (2 mM) and transform more than half amount to elemental selenium. On the other hand B.DBT1 can only convert 0.5 mM of selenite, while is able to transform completely initial 0.1 mM concentration of tellurite to elemental tellurium.



**FEMS-0618**

**Environmental microbiology - 2**

**VINASSE AS SUBSTRATE FOR GROWTH MICROBIAL CONSORTIUM-  
ALTERNATIVE FOR TREATMENT AND RE-USE WASTE.**

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**Background**

Vinasse showed to be appropriate substrate for yeast growth in single culture. The use of vinasse as a substrate for microbial biomass production can be an alternative for the treatment and reuse of this waste.

**Objectives**

The objectives of this study were (i) to evaluate the biomass production by *Bacillus subtilis* and two strains of *Saccharomyces cerevisiae* in mixed culture using the vinasse as a substrate, (ii) investigate the nutritional quality of the product generated, (iii) to evaluate the effect of biological treatment on physico-chemical parameters and level of toxicity.

**Methods**

*B. subtilis* (CCMA 0087), *S. cerevisiae* (CCMA0137) and *S. cerevisiae* (PE2) were initially grown in 100 ml vinasse medium (49% v/v vinasse, 49% distilled water, 1% glucose and 1% yeast extract). The microorganisms grown in a consortium, two by two, and each consortium was conducted in three different cell concentration. During the incubation period were monitored the cell number and pH. After that, the dry biomass and the reduction in Chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of treated vinasse was evaluated.

**Conclusions**

Based on these results, the best combination and concentration of microorganisms was selected for cultivation in bioreactor. During the incubation period the bioreactor same previous parameters were monitored. In addition to the COD and BOD, the treated vinasse was evaluated for acute phytotoxicity. The data obtained showed that the two strains of *S. cerevisiae* in mixed culture with an initial population of  $10^7$  cells/mL showed 176mg/L of biomass and reducing the power polluter of vinasse.

## DISTRIBUTION AND GENOMIC ADAPTATION OF METHANOMASSILIICOCCALES ECOTYPES IN WETLANDS AND ANIMAL INTESTINAL TRACTS

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### Background

Methane (CH<sub>4</sub>) is a very potent greenhouse gas, mainly produced by methanogenic archaea as an endproduct of their anaerobic respiration. Methanogenic archaea are phylogenetically diverse and living in a broad range of anaerobic environments, among others, in wetlands and ruminant animals, which represent the major natural and anthropogenic CH<sub>4</sub> sources. Recently, a novel order of methanogens, the *Methanomassiliicoccales*, was discovered.

### Objectives

We aimed to assess the environmental distribution of *Methanomassiliicoccales* in different wetlands (arctic and acidic/neutral temperate peat) and animal intestinal tracts. Additionally, a metagenomic characterisation of *Methanomassiliicoccales* enrichments was intended.

### Methods

Screening for *Methanomassiliicoccales* 16S rRNA and *mcrA* genes showed that *Methanomassiliicoccales* are widely distributed in wetlands and animal intestinal tracts. Phylogenetic analyses of 16S rRNA genes revealed two distinct animal and wetland clusters, pointing to two fundamentally different ecotypes. Ruminant enrichment cultures grow in an anaerobic medium supplemented with trimethylamine and H<sub>2</sub> as substrates. *Methanomassiliicoccales* represented one major methanogenic group in the bovine rumen performing H<sub>2</sub>-dependent methylotrophic methanogenesis. Two partial *Methanomassiliicoccales* genome bins assembled from rumen metagenomes represented the two ecotypes and enabled the characterisation of the underlying genomic basis of their adaptations. Ecotype differences were reflected e.g. by the smaller genome size of the animal-associated ecotype as well as a higher abundance of genes for oxidative stress response in the soil ecotype.

### Conclusions

Our findings suggest that *Methanomassiliicoccales* represent a novel ecologically important group of methanogens with distinct animal and soil associated ecotypes adapted to these different environments. Especially the animal-associated ecotype potentially plays an important role in global CH<sub>4</sub> formation.



**OZONATION FOR THE REDUCTION OF THE MICROBIAL LOAD OF WASTEWATERS**

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**Background**

Treated municipal wastewater contains microbial densities up to 10<sup>6</sup> CFU/100mL. Ozonation is an oxidation process widely used to disinfect water due to the strong ozone biocidal properties.

**Objectives**

This work aimed at assessing the effectiveness of ozonation on the reduction of the microbial load of treated wastewater.

**Methods**

A synthetic wastewater containing a mixture of fungi and bacteria, including antibiotic resistant and endospore forming strains, was submitted to ozonation assays performed in a 1L lab-scale reactor, with contact periods of 15, 30 and 60 min. The treatment efficiency was assessed based on the microbial enumeration and quantification of 16S rRNA, and selected indicator genes, before and after ozonation and after three days of storage at room temperature.

**Conclusions**

Ozonation for 15 min reduced the total heterotrophic bacteria and fungi by 3 log and 1 log, respectively. Coliforms and enterococci were extensively removed, being detected at densities of about 5 CFU/100mL. However, 3 days after water ozonation, a strong recovery was observed, with total heterotrophs reaching 10<sup>9</sup> CFU/100mL and fungi 10<sup>2</sup> CFU/100mL. Total coliforms and enterococci were still detected after 3 days. After 30 and 60 min of ozonation, viable cell counts were below the detection limit (1 CFU/100mL) and no recovery was observed after 3 days. Using culture independent methods, it was possible to confirm those results, being observed significant reductions of selected indicator genes after 15-30 min treatments. However, both 16S rRNA and *bla*<sub>TEM</sub> genes, but not *vanA*, were detected after 3 days recovery.



## MIGRATORY HABITS, AREA AND HABITAT INFLUENCE THE MICROBIOTA OF BIRDS IN THE DANUBE DELTA NATURAL RESERVE

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### Background

Wild birds from aquatic habitats could, as reservoirs and vectors for antibiotic resistant bacterial pathogens, directly affect the health of animals and pose serious risks to residents and tourists, the microbial load and its variety increasing with the broadening of the habitat and depending on the migration area.

### Objectives

The research aimed to monitor the variation and distribution of pathogenic bacteria in birds with different migratory habits, areas and habitats in the Danube Delta.

### Methods

A total of 112 rectal and oro-pharyngeal swabs from 26 species of migratory and sedentary birds from the Danube Delta Natural Reserve were collected and subjected to classical cultivation or enrichment techniques. 113 strains were identified on Chromogenic UTI medium Brilliance™ and TCBS Cholera medium (Oxoid) as belonging to *Vibrio*, *Pseudomonas*, *Proteus*, *Escherichia*, *Staphylococcus* and *Enterococcus* genera. Pathogenic bacteria were prevalent in migratory versus sedentary birds, with alarming proportions of *Vibrio spp.* (23.89%), followed by *E.coli*/coliforms (21.26%) (table 1):

Bacterial pathogens (%) isolated from cloacal and oro-pharyngeal specimens collected from wild bird species in the Danube Delta Natural Reserve (Table 1).

	V. cholerae, V. fluvialis	V. alginolyticus, V. metschnikovii	Pseudomonas spp.	V. mimicus, V. vulnificus	Proteus spp	Coliforms	Staphylococcus spp.	Enterococcus faecalis	E. coli
Wild birds									
Sedentary	11.1	37.5	37.5	30	28.57	35.7	28.57	44	30
Migratory	88.9	62.5	62.5	70	71.43	64.3	71.43	56	70

Of total strains	7.96	7.08	7.96	8.85	6.19	12.39	18.58	22.12	8.87
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## Conclusions

Thus, the bacterial load but not the spectrum of isolated species is influenced by migration site and habits rather than habitat.

**FEMS-1894**

**Environmental microbiology - 2**

## **BIODEGRADATION OF PERCHLOROETHYLENE AND TRICHLOROETHYLENE UNDER AEROBIC CONDITION**

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### **Background**

Tetrachloroethene (also known as perchloroethylene; PCE) and trichloroethylene (TCE) are highly chlorinated hydrocarbons, are the common groundwater contaminants in industrial areas. Dechlorination of hydrocarbons is a complex process and biological dehalogenation under aerobic condition also required more clarification.

### **Objectives**

The hydrocarbon degrading bacterial communities produces mono/di oxygenase enzymes and has the potential to degrade the chlorinated hydrocarbons with co-metabolic pathways. Soil samples were collected from contaminated site and enrichment of specific microbial communities were obtained using methanol and toluene as sole carbon source.

### **Methods**

The enrichment cultures Ui-mix and MEOH-1 were examined for the ability to degrade TCE and PCE under aerobic condition. Growth and degradation rate are examined using spectrophotometer and gas chromatography equipped with ECD. The colonies on the enrichment cultures are isolated, purified and identified based on the 16S rRNA gene sequencing.

### **Conclusions**

We have developed two consortiums which can degrade the PCE and TCE completely in aerobic condition. Ui-mix and monocultures from the consortium showed highest degradation effectivity to co-metabolize TCE along with toluene as sole carbon source. Similarly, the MeOH-1 and monocultures degraded PCE under aerobic condition. The consortium Ui-mix contain diverse group of bacteria includes *Chryseobacterium*, *Pseudomonas*, *Enterobacter* and *Microbacterium*; similarly *Pseudomonas*, *Hyphomicrobium*, *Cupriavidus*, and *Microbacterium* are isolated from MeOH-1. The strains M3-1, M3-3 and M3-7 showed 98.4%, 95.3% and 94.5% removal efficiency of TCE and PCE within 20 days, respectively. Further studies have to be performed to optimize and enhance the removal efficiency and it gives a gateway for the bioremediation of chlorinated ethane.





**FEMS-0724**

**Environmental microbiology - 2**

**METHANOGENIC ACTIVITY AND DISTRIBUTION OF THE  
METHANOMICROBIALES IN TRANSITIONAL BOG AND SWAMP FOREST PEAT  
PROFILES (POLESKI NATIONAL PARK, POLAND)**

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**Background**

Peatlands are the largest natural carbon storage. Under good aeration conditions carbon is released as a CO<sub>2</sub> but when there is lack of oxygen, anaerobic brake down of organic matter take place and CH<sub>4</sub> release occur. In most peatlands with neutral pH, the main substrate of CH<sub>4</sub> production is acetate while in acidic, the main precursors are CO<sub>2</sub> and H<sub>2</sub>. CH<sub>4</sub> production is attributed to the activity of methanogenic *Archaea*, which, in peat soils are represented in majority by the members of the *Methanomicrobiales*.

**Objectives**

The aim of presented work were: (1) to examine which layers of the peat soil profiles (from the surface top to a mineral base) in transitional peat bog and swamp forest surrounding eutrophic Lake Moszne in Poleski National Park (East of Poland) show the highest methanogenic activity; (2) to test the presence of the *Methanomicrobiales* in investigated peat materials.

**Methods**

Methanogens were detected in fresh samples *via* FISH technique using MG1200 probe. Soil samples were further incubated anaerobically (at 5-30°C) and CH<sub>4</sub> production (gas chromatography technique) was controlled.

**Conclusions**

The highest methanogenic activity (up to 39.46 mg CH<sub>4</sub> kg dw<sup>-1</sup> d<sup>-1</sup>) below layers 45 cm (transitional bog) and 150 cm (swamp forest) were found. In all of the investigated samples the presence of coccus, bacillus and curved rods from the *Methanomicrobiales* were confirmed. Among investigated, morphologically diverse order, the dominant role plays coccus (up to 100%) while the rarest were representatives of curved rods (up to 9%).

**FEMS-1626**

**Environmental microbiology - 2**

## **RESPONSE OF THE COALBED ROCK METHANOTROPHIC COMMUNITY TO TEMPERATURE CHANGES**

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### **Background**

Methanotrophic bacteria are ubiquitous in the environment and play an important role in the global carbon cycling. Recently published research revealed that in spite of severe oxygen limitation, aerobic methanotrophic species are also found in deep subsurface geological formations such as coalbed rocks. These rocks are excavated in huge amounts during coal exploitation and comprise a troublesome waste. The presence of the methanotrophic community creates an opportunity to reuse these materials for the purpose of methane biofiltration; however surface conditions vary much from these *in situ*, especially according to temperature, which in geological formations is stable (c.a. 30 °C).

### **Objectives**

In the current study we investigated methanotrophic communities of the rocks originating from Upper Silesian (Poland) coal mines in terms of identity (16S rRNA sequences) and response to the temperature changes.

### **Methods**

Assays were performed in hermetic microcosms where samples of 15 g of rock were incubated under mixtures of methane and air. Headspace gases were measured by means of gas chromatography. DNA was isolated using PowerSoil® DNA Isolation Kit (MoBio) and amplicons obtained with methanotroph-specific IF/IR, IIF/IIR primers.

### **Conclusions**

The investigated microbial communities were heterogeneous and consisted of both type I and II methanotrophs. Their ability of to oxidize methane decreased along with temperature and in majority, yet not all, of the samples was inhibited at 10 °C. It was hypothesized that the potential of methane turnover in the coal bed rocks is related with identity of the methanotrophic inhabitants with type II methanotrophs ( $\alpha$ -Proteobacteria) being responsible for methane oxidation at low temperatures.



**FEMS-1263**

**Environmental microbiology - 2**

**UTILIZATION OF RHAMNOLIPIDS DURING BIOSURFACTANT-MEDIATED  
BIOREMEDIATION OF HYDROCARBON-POLLUTED SOIL**

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**Background**

It was proven that rhamnolipids are environmental-friendly biosurfactants produced by *Pseudomonas* species. There are many studies dealing with rhamnolipids-mediated bioremediation of hydrocarbons. However, it must be mentioned that both the environmental fate and the influence of rhamnolipids on microbial communities remain unexplained. We hypothesize that rhamnolipids could be more readily utilized by soil microbial communities than hydrocarbons.

**Objectives**

1. to investigate and compare biodegradation rates of rhamnolipids and hydrocarbons.
2. to investigate the effect of rhamnolipids on quantitative and qualitative composition of microbial community

**Methods**

Biodegradation rates – HPLC-MS, structure of the microbial community – Illumina.

**Conclusions**

In most cases at least 80% of monorhamnolipids and dirhamnolipids were degraded during field experiment. The biodegradation rates of hydrocarbons was significantly lower compared with rhamnolipids. Rhamnolipids are more preferentially degraded by soil microorganisms than hydrocarbon compounds. Thus, we suggest that the role of rhamnolipids during bioremediation processes may be negligible.

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**FEMS-0751**

**Environmental microbiology - 2**

## **CHARACTERIZATION OF A PSEUDOMONAS SP. ISOLATED FROM BRAZILIAN SOIL AND ITS ABILITY TO DEGRADE ATRAZINE.**

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### **Background**

Atrazine is a selective and systemic herbicide used for controlling weeds in several crops. It is considered an environmental pollutant due to its high potential to contaminate the surface water and groundwater. *Pseudomonas* sp. ADP, the model strain for atrazine biodegradation, mineralizes this herbicide using it as a nitrogen source.

### **Objectives**

Characterization of a strain isolated from a Brazilian soil sample and its ability to degrade atrazine.

### **Methods**

The soil sample was collected in Ribeirão Preto, São Paulo, and processed according to the method describe by Gargouri et al. (2013), with modifications. The strain was selected from ATZ-R medium, in accordance with the formation of clear zones around the colonies. The identification was performed by 16S rRNA sequence analysis. PCR reactions for detection of *atz* genes (*atzA*, *atzB*, *atzC*, *atzD*, *atzE* and *atzF*) was carried out according to the methodology of Devers et al. (2004) and, then, sequenced. Plasmidial DNA extraction was performed according to the alkaline lysis technique, previously described by Birboin & Doly (1979). The degradation test was performed in solid and liquid ATZ-R medium.

### **Conclusions**

The strain was identified as *Pseudomonas* sp., presented all *atz* genes and a 35 MDa plasmid, however, only the *atzA* gene was detected in this plasmidial DNA. The strain was capable to mineralize this herbicide after 48 hours in solid medium, indicated by the clear zones around the colonies. The analysis of the ATZ-R liquid medium in spectrophotometer (225 nm) showed a significant decrease of atrazine after 48 hours of incubation.

**THE MICROORGANISM THAT NEVER READ THE LITERATURE - FASTEST GROWING PHOTODAMAGE TOLERANT ALGA ISOLATED FROM DESERT CRUSTS, DEPENDS ON PIONEER FILAMENTOUS CYANOBACTERIA SPECIES TO SURVIVE DESICCATION**

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**Background**

With the premise that unique capabilities can be found in microorganisms facing extremely harsh conditions, we focused our efforts on desert biological sand crusts (BSC), one of the harshest environments to support life. Recently, we have isolated a small green alga (named *Chlorella ohadii*), which does not conform to some of the basic fundamentals in microbial ecology and photosynthesis:

**Objectives**

1. Acquired ability to acclimate to extreme environments is usually accompanied by reduced performance under optimal conditions. We intend to show that *C. ohadii* **does not obey this rule**. In its natural habitat, *C. ohadii* is facing diurnal desiccation-hydration cycles, vast temperature amplitudes and extremely high illumination intensities, yet when grown under optimal laboratory conditions it exhibits the fastest growth rates ever reported for an alga.

2. After many years of research, some feel that we have elucidated the functioning of the photosynthetic machinery, and what sets the upper limit for algal growth. However, the unparalleled fast growth, extremely high photosynthetic rates and resistance to photodamage, suggest this **may not be the case**. Namely, *C. ohadii* has been shown to be completely resistant to photoinhibition, and its productivity was unaffected by irradiances as high as twice full sun light.

3. Survival of *C. ohadii* in its BSC habitat depends on close association with filamentous cyanobacteria; **a unique and novel mode of interspecies interaction**.

**Methods**

We wish to present detailed physiological analysis of the unique properties supporting this phenomenal growth and resistances, combined with insights from their genomic, transcriptomic and metabolomic characterization.

**Conclusions**



Treves, H., Raanan, H., Finkel, O., Berkowicz, S., Keren, N., Kaplan, A. (2013) A newly isolated *Chlorella* sp. from desert sand crusts exhibits a unique resistance to excess light intensity. FEMS Microbiology Ecology. doi:10.1111/1574-6941

## **DETERMINING THE EFFECT OF SAMPLING VOLUME AND FLOW RATES ON INVESTIGATIONS OF BACTERIAL COMMUNITY DIVERSITY IN A LOW BIOMASS AQUATIC ENVIRONMENTS**

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### **Background**

As water travels from treatment plant through the pipelines to consumer's taps, the diverse drinking water microbiome varies in abundance and in composition as a result of a large number of variables. A quantitative understanding of relevant variables is essential towards ensuring better control of drinking water quality. Increasingly, DNA sequencing approaches such as 454 pyrosequencing and Illumina MiSeq sequencing are being utilised to this end. Yet, little has been done to understand the influence of sample collection protocols on the data collected through DNA sequencing approaches. The ability to accurately understand the factors that affect the change in the drinking water microbiome is critically reliant on the robust approaches for sample collection.

### **Objectives**

The goal of this study was to understand the effect of sample volume and sampling flow rate on the structure and membership of drinking water bacterial communities. Understanding these effects is especially critical in drinking water systems, which represents a low biomass but high diversity aquatic environment.

### **Methods**

To do this, we sampled at five different residential sampling locations in the City of Glasgow, UK. This sampling was conducted such that five different sample volumes, ranging from 1 to 20 litres, were filtered to harvest microbial cells at each sampling location and this exercise was repeated at two different flow rates at the faucet (laminar and turbulent flow regimes).

### **Conclusions**

We will provide a detailed overview of these two variables on the reproducibility and reliability of DNA sequencing based investigations of the drinking water microbiome.

**PHYSIOLOGIC AND GENOMIC CHARACTERIZATION OF A NOVEL NITROSPIRA SPECIES ENRICHED UNDER ANAEROBIC, DENITRIFYING CONDITIONS**

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**Background**

Members of the genus *Nitrospira* are dominant in many natural habitats and of vital importance for wastewater treatment. They are chemolithoautotrophic organisms capable of growth with nitrite and CO<sub>2</sub> as sole energy and carbon source. It further has been demonstrated that some *Nitrospira* can utilize simple organic carbon compounds and molecular hydrogen as alternative substrates and can switch to nitrate reduction under oxygen limitation. However, this metabolism has been assumed to be a survival strategy and in most systems studied so far *Nitrospira* was outcompeted by denitrifying organisms when anoxic conditions prevailed.

**Objectives**

Here, a community of nitrogen cycle bacteria was enriched from the anaerobic compartment of a biofilter connected to a recirculating aquaculture system.

**Methods**

The culture was fed with filtered water from the aquaculture system, supplemented with ammonium, nitrite and nitrate.

**Conclusions**

A stable enrichment culture was obtained which anaerobically converted ammonium, nitrite and nitrate into dinitrogen gas. The culture was dominated by anaerobic ammonium-oxidizing *Brocadia* species, a denitrifier (*Denitratisoma*), and a *Nitrospira* species. Anammox bacteria and *Nitrospira* co-aggregated, while the denitrifiers formed separate clusters. This culture shows that anaerobic ammonium oxidizers and nitrite oxidizers, who were considered to be mutually exclusive, can be grown as a stable co-culture. Furthermore, this shows that some *Nitrospira* species can be competitive under nitrate-reducing conditions. This study demonstrates another unexpected lifestyle for an organism believed to be only competitive under aerobic lithoautotrophic conditions. It further indicates that *Nitrospira* in combination with anammox can be of interest for anoxic wastewater treatment systems.

**FEMS-1853**

**Environmental microbiology - 2**

## **OXALATE-DEGRADING ABILITY IS UBIQUITOUS IN DIAZOTROPHIC AZOSPIRILLUM**

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### **Background**

Oxalate-degrading bacteria are known from a wide variety of ecological niches and different taxonomical origins. They play important roles in local and global carbon-cycling and, when present in the guts of mammals, they seem to provide protection against excess oxalate uptake and renal stones.

### **Objectives**

In the present study, we aim to investigate the ability to grow at minimal medium with Ca-oxalate or Na-oxalate as a sole carbon source of 30 diazotrophic *Azospirillum* strains isolated from the rhizosphere of various field grown gramineous plants species grown at different geographical Greek locations, molecular characterized at our previous research works, and reference type strains. Most of these strains tested for the ability to grow at minimal medium with oxalate crystals isolated from *Amaranthus blitum* (family *Amaranthaceae*) as a sole carbon source. Additionally, we investigated for the presence of formyl-CoA transferase (*frc*) gene a molecular marker used for the specific detection and identification of oxalotrophic bacteria involved in oxalate degradation.

### **Methods**

Minimal media with Ca-oxalate/Na-oxalate or oxalate crystals from plant were used as a sole carbon source for bacterial growth. Molecular biology techniques and phylogenetic analysis for the *frc* genes.

### **Conclusions**

The majority of the examined strains display an ability to use oxalic acid as a sole carbon source. This result and the presence of the *frc* genes shows that these strains can metabolize oxalate via the oxalyl - CoA - decarboxylase pathway to produce formate and incorporate it in their metabolism.

**FEMS-1898**

**Environmental microbiology - 2**

**DETOXIFICATION AND BIODEGRADATION OF ENVIRONMENTAL  
POLLUTANTS BY MARINE MICROBIAL CONSORTIA**

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**Background**

The high industrial activity causes several environmental problems, mainly due to the discharge of wastes. Marine environments are susceptible to contamination by industrial waste and also may represent a target niche for microbial prospecting for bioremediation. Marine microorganisms are adapted to saline conditions and have potential for being used in many processes.

**Objectives**

To evaluate the potential of microbial consortia in detoxify and degrade environmental pollutants.

**Methods**

Microbial consortia were structured in different combinations using four ligninolytic fungi from marine environments, two bacteria from oil reservoir and two lipolytic yeasts from Antarctica, previously selected based on their capacity to produce enzymes and to degrade hydrocarbons. Erlenmeyer flasks containing 50 ml of mineral medium, the microbial consortium and the pollutant (RBBR 500 ppm and Diesel oil 1% vv) were kept in incubators for 7 (RBBR), 14 and 21 (diesel oil) days at 140 rpm and 28 °C. Samples were analyzed in Microtox for acute toxicity and ligninolytic enzymes (Lac, MnP, LiP) were quantified.

**Conclusions**

Consortium 7 showed promising results in the studies with RBBR dye, presenting discoloration up to 70% and a very low rate of mycelial adsorption (11%). Enzymatic production of Lac was up to 141 U/L. LiP and MnP production were very low. All samples containing RBBR were detoxified. Samples containing diesel oil presented no detoxification and the production of ligninolytic enzymes was higher for MnP (114 U/L). A longer period of incubation and/or a lower concentration of oil will be tested, since this compound is highly complex.

**FEMS-1578**

**Environmental microbiology - 2**

## **EFFECTS OF DUST STORM EVENTS ON TUBERCULOSIS INCIDENCE RATE IN NORTHWEST OF CHINA**

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### **Background**

**Tuberculosis (TB) is a major public health problem in China. Gansu has very high annual attendance rate of TB in China, and the province is also famous because of its severe dust storms. The epidemic timing starts in February and ends in July, and the dust storm mainly distribute throughout the spring and early summer, which strongly indicate a close linkage between causative agent of TB and dust storm events.**

### **Objectives**

We investigated the general impact of dust storms to TB across time by analyzing variation in weekly clinic visits in Gansu during 2005-2012 in relation with the climatic event.

### **Methods**

We applied Mann-Whitney-Pettitt test and regression model to detect the seasonal periodicity of TB and dust storm in a time series, and assess relationships between the meteorological variables and weekly TB clinic visits.

### **Conclusions**

By comparing the information on cases of TB of Gansu weekly reported with dust storm events, we found a clear link between population dynamics of the disease and the climate disaster: the onset of epidemics and the dust storm shared the almost same mean week. Particulate matter might be the direct reason for the outbreak of TB in dust storm days. To our knowledge, this is the first population-based study that provides a clear demonstration that epidemic of TB was affected by dust storm in China, which will help understanding the association between this environmental problem and the evolution of epidemic disease.



**FEMS-0620**

**Environmental microbiology - 2**

**GENETIC INSIGHTS INTO THE SULFUR OXIDATION PATHWAY OF SULFUR CHEMOLITHOTROPHS LIVING IN FRESHWATER LAKES**

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**Background**

Chemolithotrophic sulfur-oxidizing bacteria are capable of oxidizing inorganic sulfur compounds as sources of energy for growth. A number of genomic studies have been taken place focusing on sulfur oxidizers living in habitats where a sufficient amount of reduced inorganic sulfur compounds are available. These studies revealed that taxonomically diverse sulfur chemolithotrophs use different enzymes for the oxidation of various sulfur compounds. On the other hand, sulfur chemolithotrophs living in freshwater lakes, where the availability of reduced inorganic sulfur compounds is generally low, have been overlooked. Recently, we revealed the dominance of betaproteobacteria in the sulfur-oxidizing bacterial community of freshwater lakes, and isolated several of them in pure culture.

**Objectives**

The aim of this study is to gain genetic insights into the sulfur oxidation pathway of sulfur chemolithotrophs isolated from freshwater lakes.

**Methods**

The genomes of the new isolates were sequenced and then analyzed focusing on the composition and phylogeny of genes for sulfur oxidation. In addition, their genomes were compared with available complete genomes of other sulfur oxidizers.

**Conclusions**

As the first comprehensive comparative genomic analysis focused on sulfur chemolithotrophs from freshwater lakes, the results of our study revealed that they possess common sets of genes putatively involved in sulfur oxidation. One of these gene sets was shared only by freshwater sulfur-oxidizing betaproteobacteria.



**FEMS-1762**

**Environmental microbiology - 2**

## **NITROTOGA - A MAIN NITRITE OXIDIZER IN LOW TEMPERATURE ENVIRONMENTS**

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<sup>2</sup>Microbiology, Radboud University Nijmegen, Nijmegen, Netherlands

### **Background**

*Candidatus Nitrotoga arctica* is a cold adapted nitrite-oxidizing bacterium (NOB), which was cultivated from the Siberian Arctic. Since 2007, *Nitrotoga*-like NOB could be detected in different natural and technical environments e.g. beneath the Antarctic ice sheet, in cave systems, in wastewater treatment plants and in aquaculturing facilities. Thus, they are more distributed than previously assumed and of importance for the global nitrogen cycle as well as for a successful nitrification in wastewater processing.

### **Objectives**

The aim of this project is to gain a deeper understanding of the phylogenetic diversity within the genus *Nitrotoga* belonging to the *Betaproteobacteria* and to compare representatives derived from natural and artificial ecosystems.

### **Methods**

The 16S rRNA gene was analysed combining specific and semi specific primer pairs for Eubacteria and *Nitrotoga*. Additionally, the beta subunit of the key enzyme of nitrification in *Nitrotoga* and other NOB, the nitrite oxidoreductase B (nxrB), was sequenced.

### **Conclusions**

So far, *Nitrotoga*-like bacteria from widespread habitats do not differ noticeably in their 16S rRNA gene sequence from each other, not even those from extreme or technical origin (similarity > 98%). Although they might possess various physiological features, different species cannot be recognized on basis of the 16S rRNA gene. A higher resolution was obtained by comparing the sequences of the gene *nxrB*. In contrast to the ubiquitous and phylogenetically ancient NOB genus *Nitrospira*, *Nitrotoga* does not seem to be very diverse so far. However, its high abundance in various environments makes it an important player in nitrification.



**FEMS-2748**

**Environmental microbiology - 2**

## **NAPHTHALENE DEGRADATION BY SULFATE-REDUCING MARINE COMMUNITIES**

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### **Background**

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitously distributed to the environment through the extensive petroleum and chemical industries. These toxic chemicals are of concern because of continual, widespread release.

### **Objectives**

The bicyclic hydrocarbon naphthalene undergoes aerobic and anaerobic transformations, however our understanding of the details of anaerobic naphthalene degradation lags behind that of aerobic processes.

### **Methods**

Sediment was obtained from Tuckerton, NJ, where PAHs are introduced through nonpoint deposition to an otherwise relatively pristine ecosystem. Anaerobic enrichment cultures were established with 10% anoxic estuarine sediment under sulfate reducing conditions and amended with 500µM naphthalene as the sole carbon source. Naphthalene concentrations were monitored using GC/FID.

### **Conclusions**

Naphthalene loss was observed in 139 days in primary enrichments, followed by 48 days in transfer cultures. Subsequent transfers receiving 30-50% active cultures are sediment free and degrade naphthalene within 21 days. Stoichiometric loss of 3mM sulfate per 500µM naphthalene is observed. In the presence of molybdate, which inhibits sulfate reduction, no naphthalene degradation occurs, linking sulfate reduction to naphthalene metabolism. Distinct bands unique to naphthalene degrading cultures are seen in 16S rDNA analyzed by DGGE, indicating the enrichment of naphthalene degrading bacteria. By incorporating <sup>13</sup>C-Naphthalene into DNA and RNA via stable isotope probing (SIP), the active microbes responsible for naphthalene degradation can be determined. Additionally, the recovery of 2-naphthoyl-CoA-reductase from <sup>13</sup>C-DNA as well as identification of <sup>13</sup>C intermediates can help to describe the metabolic pathway. This investigation of a robust sulfidogenic marine enrichment culture advances our understanding of the diversity of microbes able to anaerobically degrade naphthalene.

**FEMS-0714**

**Environmental microbiology - 2**

**QUANTIFICATION OF SOIL MICROBIOLOGICAL ACTIVITIES BASED ON CROP TYPE (OAT, TRITICALE)\***

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<sup>3</sup>Microbial Biochemistry, Warsaw University of Life Sciences, Warsaw, Poland

**Background**

Agriculture in Poland has been an important part of the country's economy (about 60% of Poland's total area is used for crop cultivation). It is known, that agricultural types of soil contain much smaller number of microorganisms, in comparison with natural soils. Thus we hypothesised that agricultural soils are biologically degraded and not being able to become naturally regenerated which may lead to their inability of regaining the satisfactory level of fertility.

**Objectives**

The aim of the study was to indicate which type of crop: oat or triticale is more favourable for sustaining soil microbiological activity.

**Methods**

Eight different soil units agriculturally used, and the same number of control soils not agriculturally exploited were studied. Soils were collected in April 2014, using Egner's bow from the surface layer. Under laboratory conditions the following analyses were performed: pH, electric conductivity, total carbon, phosphorus and nitrogen content, microbial biomass (chloroform fumigation), respiration (GC), soil dehydrogenase activity (TTC) and DNA isolation (chloroform extraction method with CsCl<sub>2</sub> gradient). Spearman's rho correlation coefficient was used to assess relationships between chemical and microbiological soil properties.

**Conclusions**

It was demonstrated that cultivated soils are biologically degraded, what was evidenced by lower values of all microbiological parameters in relation to control sites. Comparing crop type we found that triticale is more favourable for sustaining soils microbiological activity rather than oat. Determined positive correlations proved that pH, carbon, phosphorus and nitrogen content are the most important factors determining soil microbiological activities.

*\*Project was financed by the National Science Centre (Poland), granted on the basis of decision DEC-2013/09/D/NZ9/02482.*



**FEMS-0718**

**Environmental microbiology - 2**

## **ENVIRONMENTAL FACTORS INFLUENCED ON SOIL MICROBIOLOGICAL ACTIVITY\***

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### **Background**

Many of recent studies have focused on documenting how soil bacterial communities are affected by specific environmental changes or disturbances. It was indicated that vegetation type, carbon and nutrient availabilities, and soil moisture may influence microbial activity at local scales, whilst soil pH seemed to be a better predictor at the continental scale.

### **Objectives**

The aim of the study was to determine effect of selected environmental factors: pH, redox potential (Eh) and total carbon (TC) on soil microbiological activity, expressed as microbial biomass (MB), DNA content, potential ammonification (PA) and most probable number (MPN) of ammonifying bacteria.

### **Methods**

Fifth representatives of *Eutric Cambisols* collected in April 2014 from the surface layer of agricultural sites were investigated. Spearman's rho correlation coefficient was used to determine relationships between environmental and microbiological soil properties.

### **Conclusions**

Positive correlations among pH, TC and all microbiological properties were reported. pH was found to be proportionally related with MB (0.73\*\*), DNA (0.84\*\*\*), PA (0.65\*\*) and MPN (0.73\*\*). Analogical trend demonstrated TC, what was confirmed by values of r coefficients, as follows: MB (0.64\*\*), DNA (0.78\*\*), PA (0.76\*\*), MPN (0.83\*\*\*). In the case of Eh it was noted that its lower values have stimulating effect on microbiological activity in *Eutric Cambisols*, as evidenced by following assigned rho: MB (-0.69\*\*), DNA (-0.83\*\*), PA (-0.75\*\*) and MPN (-0.82\*\*). Among investigated environmental factors only impact of moisture have insignificant character ( $p > 0.05$ ) in relations to tested soil microbiological properties.

*\*Project was financed by the National Science Centre (Poland), granted on the basis of decision DEC-2013/09/D/NZ9/02482.*

**FEMS-3047**

**Environmental microbiology - 2**

**THERMOPHILIC METHANOGENS REDUCE AMORPHOUS FE(III) OXIDES AND GENERATE CRYSTALLINE MAGNETITE ON CELL SURFACES**

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**Background**

Methane production is affected by various environmental stimuli including the presence of Fe(III) oxides [1,5]. Inhibitory effect of Fe(III) oxides and Fe(III)-reducing ability in mesophilic methanogens has been reported [2,3]. There have been only limited information about thermophilic methanogens.

**Objectives**

Thermophilic methanogens were investigated for their ability to reduce poorly crystalline Fe(III) oxides (ferrihydrite) and the inhibitory effects of ferrihydrite on their methanogenesis.

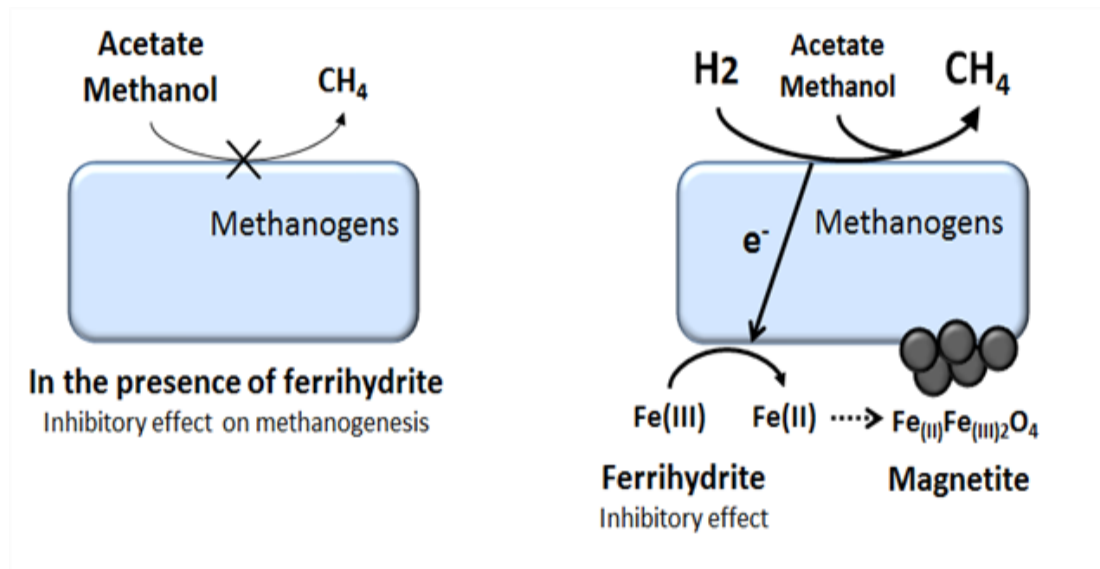
**Methods**

*Methanothermobacter thermautotrophicus* strain  $\Delta H^T$ , *Methanosaeta thermophila* strain  $PT^T$ , and *Methanosarcina thermophila* strain  $TM-1^T$  and strain FE-1 were anaerobically cultivated in various methanogenic substrates supplemented media at 55 °C in the presence or absence of ferrihydrite. The amounts of methane and concentrations of Fe(II) were measured by gas chromatography and ferrozine method, respectively.

**Conclusions**

Fe(III) reduction was observed by the thermophilic methanogens only in the presence of  $H_2$  as the reducing source [4]. While addition of ferrihydrite resulted in inhibition of methanogenesis, ferrihydrite reduction by the methanogens partially alleviates the inhibitory effects. *Methanosarcina thermophila* generated magnetite particles on its cell surfaces through ferrihydrite reduction. These findings suggest that interaction between Fe(III) minerals and methanogens has significant impacts on materials and energy cycles in anoxic environments.





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**FEMS-0788**

**Environmental microbiology - 2**

## **OPPORTUNISTIC PATHOGENIC YEASTS ON THE SURFACE OF FRUITS**

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### **Background**

Infections caused by treatment-resistant non-albicans *Candida* species, such as *C. tropicalis*, have increased. It has become an emerging challenge in managing fungal infections. Previous researches have showed that *C. tropicalis* are opportunistic yeasts exist in environment.

### **Objectives**

We are interested in whether the opportunistic yeasts may spread from environment into human body.

### **Methods**

We isolated and characterized pathogenic yeasts on the surface of fruits from supermarkets. A total of 291 isolates of 83 species from 24 different types of fruits were recovered. We have determined their species and drug susceptibility.

### **Conclusions**

Of the 83 species, 7 common pathogenic *Candida* species were detected. They included 16 *C. guilliermondii*, 15 *C. famata*, 3 each of *C. parapsilosis* and *C. tropicalis*, 2 each of *C. krusei*, *C. lusitaniae* and *C. orthopsilosis*. The drug susceptibilities of 162 of the 291 isolates were determined. Totally, 158 (97.5%), 104 (64.2%), and 102 (63%) isolates were susceptible to amphotericin B (MICs  $\leq$  4 mg/l), fluconazole (MICs  $\leq$  8 mg/l), and triadimenol (MICs  $\leq$  8 mg/l), respectively. One *C. tropicalis* isolate (F91) from wax apple had MICs at 64 mg/l for both fluconazole and tridimenol. It belongs to diploid sequence type (DST) 149, a genotype found in isolates from human as well as soil. Hence extra caution shall be taken when providing fruits or juice to severely immunocompromised patients since drug resistant pathogenic yeasts may be on the surface of fruits.

**FEMS-2433**

**Environmental microbiology - 2**

## **BIOREDUCTION OF TELLURIUM AND SELENIUM OXYANIONS BY UNACCLIMATED ANAEROBIC GRANULAR SLUDGE**

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### **Background**

Microbial reduction of chalcogen oxyanions such as tellurite and selenite is promising for development of innovative biotechnological processes for applications in bioremediation, wastewater treatment and production of functional nanomaterials.

### **Objectives**

Here, we demonstrate rapid and stable bioreduction of tellurite, for the first time, using unacclimated anaerobic granular sludge, obtained from an operating upflow anaerobic granular sludge blanket reactor treating paper mill wastewater. In addition, bioreduction of tellurite in the presence of selenite was determined.

### **Methods**

Bioreduction experiments were performed in serum bottles under different growth conditions using anaerobic granular sludge as source of microorganisms. Bioreduction of selenium and tellurium oxyanions profiles were determined at regular time intervals. Biochemical and microbial characterization of selenium and tellurium oxyanions reducing granular sludge was determined.

### **Conclusions**

Tellurite was rapidly reduced to brown and then to black colored elemental tellurium by anaerobic granular sludge. The reduction of tellurite occurred at a much faster rate than the selenite when supplied individually. Complete reduction of 1 mM of tellurite and selenite was observed in 8 and 96 h, respectively. When both tellurite and selenite were present in the medium, the reduction of tellurite was not significantly affected. But, selenite reduction was found to be slower in the presence of tellurite. It is interesting to note an overlap between the reduction of both tellurite and selenite, which is needed for production of Se-Te functional nanomaterials. Repeated bioreduction of 1 mM of tellurite was sustainable during multiple cycles of feeding in a fed-batch experiment, indicates the applicability in biological treatment and formation of Te based nanomaterials.



**FEMS-2494**

**Environmental microbiology - 2**

**MICROBIAL ASSOCIATIONS RELATED TO METHANE PRODUCTION IN AN UP-FLOW ANAEROBIC SLUDGE BLANKET TREATING MOLASSES WASTEWATER**

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**Background**

Anaerobic digestion of organic wastes involves complex microbial interactions. The understanding of microbial interactions in anaerobic digestion can be utilized to construct strategies for methane production stably and effectively.

**Objectives**

In this study, methane production was evaluated in the up-flow anaerobic sludge blanket (UASB) reactor using molasses wastewater, and the active bacterial and archaeal communities were characterized using rRNA-based ion torrent sequencing.

**Methods**

The UASB reactor achieved a stable process performance at an organic loading rate of 1.7~13.8 g-COD·L<sup>-1</sup>·d<sup>-1</sup> (87–95% COD removal efficiencies), and the maximum methane production rate was 4.01 L-CH<sub>4</sub>·L<sup>-1</sup>·d<sup>-1</sup> at 13.8 g-COD·L<sup>-1</sup>·d<sup>-1</sup>. Community analysis revealed that bacterial and archaeal communities shifted along with OLR, and *Lactococcus* and *Methanosaeta*, comprising up to 84% and 80% of the respective communities, drove the changes. Microbial network analysis was applied to identify associations among the relative abundances of bacterial and archaeal operational taxonomic units, environmental parameters, and reactor performance. The constructed network (average shortest path, 2.261; clustering coefficient, 0.514) revealed the prevalence of bacteria–archaea associations (50% of microbe–microbe associations) and contained seven microbial hub nodes including both bacterial and archaeal nodes. Interestingly, the *Lactococcus* and *Methanosaeta* were network hub nodes and positively correlated. In addition, they shared the other microbial hub nodes as neighbors and were positively correlated with methane production.

**Conclusions**

The results indicate that the close association between *Lactococcus* and *Methanosaeta* is responsible for the stable production of methane in the UASB reactor using molasses wastewater.

**FEMS-1686**

**Environmental microbiology - 2**

## **INFLUENCE OF SOIL PHYSICAL AND CHEMICAL PROPERTIES ON FUNGAL AND BACTERIAL COMMUNITIES IN 12 VINEYARD SOILS FROM NORTHERN ITALY**

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### **Background**

Soil chemical and physical properties, which are determined both by pedo-climatic factors and by management, can significantly influence microbial communities.

### **Objectives**

Scope of this work was to assess whether given features can specifically influence defined microbial taxa in a man-managed environment such as the vineyard.

### **Methods**

Soil samples were collected in 12 vineyards located in two neighbouring groups of hills, both renowned in Italy for their wines: the Euganean Hills, whose soils are of prevalent volcanic origin, mainly from trachyte and riolite rocks, and the Berici Hills, with a marine sedimentary genesis, mainly from clay-limestone. Soil bacterial and fungal communities were determined through 16S and ITS 454 pyrosequencing analysis, their relative taxa abundances were calculated and their levels of correlation with soil characteristics were tested.

### **Conclusions**

In order of abundance, the dominant bacterial taxonomic groups across all samples were Actinobacteria, Alphaproteobacteria, Acidobacteria, Bacteroidetes, Betaproteobacteria, Chloroflexi, Gemmatimonadetes, Planctomycetes,

Deltaproteobacteria, Gammaproteobacteria. Among fungal phyla, Ascomycota largely dominated followed by Basidiomycota. Prevailing fungal classes were Dothideomycetes, Sordariomycetes, Agaromycetes, Leotiomycetes, Eurotiomycetes and Tremellomycetes. Some of these taxa and less abundant microbial groups were found to be significantly influenced by soil pH and texture. Moreover, a considerable number of significant correlations were found between the relative abundance of microbial taxa and the amount of several different chemical parameters such as total carbon, nitrogen, phosphorus, exchangeable bases and microelements.

This study provides novel insights into how soil structure and management can affect soil microbial community composition.

FEMS-1386

Environmental microbiology - 2

## EFFECT OF BIOAUGMENTATION WITH BACTERIAL STRAINS ISOLATED FROM THE SOIL OF RICE FIELDS ON COMPOSTING PROCESSES USING RICE STRAW AND SEWAGE SLUDGE

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### Background

Composting is a biotransformation process in which solid organic matter turns into mature and stabilized material by the action of microorganisms in an aerobic process. Microorganisms play a key role in composting; the performance of the process and the quality of the final product depends on the quantitative and qualitative composition of microbial communities associated to the different stages of the process.

### Objectives

In this work, we describe the use of two bacterial strains, *Paenibacillus barcinonensis* and *Bacillus* sp. BP7, isolated from the soil of rice fields in the Ebro river delta in Spain, as bioaugmentants in composting processes using rice straw and sewage sludge as starting material.

### Methods

Composting piles were created by blending sewage sludge and rice straw at a ratio of 2.6:1 (w/w fresh weight) with a moisture content of 60%. To test the effect of the two strains, individual piles were seeded with 10<sup>8</sup> ufc/gram of either *P. barcinonensis* or *Bacillus* sp. BP7, and a series of parameters were determined during the composting process

### Conclusions

Among the parameters determined, the humification index after 25 days had increased 40% in the piles supplemented with *P. barcinonensis* and 20% in those supplemented with *Bacillus* sp. BP7, a clear indicator of the efficiency of the bioaugmentation. This was also accompanied, in the case of *P. barcinonensis*, by a change in the humic/fulvic acids ratio that went from 3.2 after 25 days in the control pile to 5.2 in the case of *P. barcinonensis*. These results suggest the potential application of bioaugmentation in composting.



**FEMS-1393**

**Environmental microbiology - 2**

## **ISOLATION OF IBUPROFEN DEGRADING BACTERIAL STRAINS FROM COMPOSTING PILES MADE UP OF RICE STRAW AND SEWAGE SLUDGE**

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### **Background**

Pharmaceutical and personal care products (PPCPs) discharged with wastewater treatment plant effluents are an emerging surface water quality concern. Ibuprofen is the most widely used member of a diverse class of pharmaceuticals termed non-steroidal anti-inflammatory drugs (NSAIDs), many of which share a phenylacetic acid (PAA) core. Ibuprofen has been detected in bodies of water worldwide. Investigations into its environmental impact have found that ibuprofen induced changes on fish, plants, algae and microbial aquatic communities at environmentally relevant concentrations.

### **Objectives**

The aim of this research was the isolation of bacteria with characteristics for potential bioaugmentation to enhance ibuprofen degradation in wastewater treatment plants and in the sewage sludge resulting from the activity of these plants.

### **Methods**

Composting piles spiked with ibuprofen were created by blending sewage sludge and rice straw at a ratio of 2.6:1 (w/w fresh weight) with a moisture content of 60% to which a concentration of 1g/l of ibuprofen was added. Samples were taken at different days in the different phases, up to 42 days when ibuprofen had been completely degraded. Microorganisms extracted from these samples were tested in minimal medium supplemented with 500 mg/ml of ibuprofen and incubated at 24°C or 55°C, to discriminate mesophilic from thermophilic microorganisms.

### **Conclusions**

Ibuprofen degradation occurred only in the samples incubated at 24°C. Plating of microorganism from these samples allowed the isolation of some 30-40 different colony morphologies that were again tested for ibuprofen degradation. Two of the isolates were confirmed to degrade ibuprofen and were partially characterized as belonging to the genus *Klebsiella*.

**FEMS-1414**

**Environmental microbiology - 2**

**DEVELOPMENT OF A TEST FOR THE DETECTION OF BACILLUS THURINGIENSIS VAR KURSTAKI IN ENVIRONMENTAL SAMPLES**

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**Background**

*Bacillus thuringiensis* was isolated for the first time in 1902 from diseased silkworm larvae and its commercial use as an ecological insecticide started in 1958. *B. thuringiensis* var *kurstaki* (*Btk* for short) is routinely used to control caterpillar pests both at large extensions and at small gardens. The crystal toxin is only active when ingested by the caterpillars and absorbed in the alkaline digestive tract. The toxin has a relatively narrow spectrum of action and does not affect mammals birds or other insects and besides, the persistence of *Btk* on foliage is of around five days and does not accumulate on the environment. Because of its relative safety *Btk* has also been in use as a simulant by armed and security forces in exercises of response to biological or bioterrorist attacks.

**Objectives**

In this work we have developed rapid tests that can be used for the detection of the presence of *Btk* on air or surface samples after controlled release, and to measure the efficiency of decontamination measures.

**Methods**

Polyclonal antibodies were raised in rabbits by injecting them with autoclaved commercial *Btk* spores.

**Conclusions**

Immune response was confirmed by Western-Immunoblot and immunofluorescence assays and the specific IgGs, after purification by affinity chromatography using *Btk* spores, were coupled to polystyrene beads, to develop a slide agglutination test, colloidal gold, as part of lateral flow immunochromatography tests, or polystyrene ELISA plates, to develop ELISA based assays. Preliminary results confirm the validity of slide agglutination test whilst lateral flow and ELISA are under development.

**FEMS-2165**

**Environmental microbiology - 2**

## **MOLECULAR IDENTIFICATION OF MICROORGANISMS USED FOR „BACTERIOGRAPHIE“**

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### **Background**

The knowledge that bacteria use pigments to guard against UV radiation gave Erich Schopf the idea of using bacteria as unique tool for creating colors. Instead of painting artists' colors on different painting supports, he applies bacteria on agar-based media. Initially invisible, the picture gets visible due to the growth of the bacteria used. Erich Schopf has called this technique 'Bacteriographie' ([www.bacteriographie.com](http://www.bacteriographie.com)).

Meanwhile the ensemble of bacteria used for 'Bacteriographie' comprises around 900 microorganisms from different regions of the world (e.g. Austria, Germany, Greenland, Iceland, India, Indonesia, Ireland). They were collected from air, food, water, soil and recently also from holy water. Next to their ability to form pigments a general criterion is their growth at room temperatures within a foreseeable time period. Additionally, they have to undergo strict screening tests to perceive their suitability.

### **Objectives**

Although some classical microbiological tests were done to get more information about the microorganisms themselves, the identity of the isolates is generally not known. Thus, this study focused on their identification at species-level using molecular biological methods.

### **Methods**

A subset of the most important isolates (n = 120) was identified by 16S rRNA gene sequencing.

### **Conclusions**

About 40 different genera could be identified. The relation between gram-positive and gram-negative species was nearly balanced. However, also a few fungal species (e.g. *Pseudozyma* spp., *Cryptococcus* spp.) were identified. Different species of the genera *Pseudomonas*, *Flavobacterium*, *Microbacterium*, *Rhodococcus*, *Kocuria*, *Chryseobacterium* and *Arthrobacter* were frequently detected, which form yellow, orange and red pigments.

**FEMS-0910**

**Extreme environments**

**RAPID RECOVERY OF CYANOBACTERIAL PIGMENTS IN DESICCATED BIOLOGICAL SOIL CRUSTS FOLLOWING ADDITION OF WATER**

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**Background**

Cyanobacteria in biological soil crusts were shown to move upwards in to track water, however this it is not known how widespread this process is in other crusts.

**Objectives**

To examine soil surface colour change to green and hydrotaxis following addition of water to crusts

**Methods**

We used pigment extraction, hyperspectral imaging, microsensors and <sup>13</sup>C labelling experiments coupled to matrix-assisted laser desorption and ionization time of flight-mass spectrometry (MALD-TOF MS)

**Conclusions**

The topsoil colour turned green in less than 5 min following water addition. The concentrations of chlorophyll *a*, scytonemin and echinenon rapidly increased in the top <1 mm layer while in the deeper layer their concentrations remained low. Hyperspectral imaging showed that, in both wet and dehydrated crusts, cyanobacteria formed a layer at a depth of 0.2-0.4 mm and this layer did not move upward after wetting. <sup>13</sup>C labelling experiments and MALDI TOF analysis showed that Chl *a* was already present in the desiccated crusts and *de novo* synthesis of this molecule started only after 2 days of wetting due to growth of cyanobacteria. Microsensor measurements showed that photosynthetic activity increased concomitantly with the increase of Chl *a*, and reached a maximum rate approximately 2 hours after wetting. We conclude that the colour change of soil crusts to green upon water addition was not due to hydrotaxis but rather to the quick recovery and reassembly of pigments.

**FEMS-0970**

**Extreme environments**

## **GEOMICROBIOLOGY OF JAZAN SABKHA, SAUDI ARABIA**

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### **Background**

Sabkha is a unique environment of economic and geologic importance with a valuable resource of distinctive features. Saudi Arabia possesses coastal Sabkhas which are extensively distributed along both the eastern and western areas.

### **Objectives**

Therefore, samples (I and II) with depth of 50 cm were collected from a remote salt marsh at the southwest of Saudi Arabia, which is called Jazan Sabkha, to unveil the bacterial community that survive in such extreme environment.

### **Methods**

Each sample was cut horizontally at interval of 1 cm for the first 10 cm and at 5 cm for the next 20 cm. The samples are considered salty and sulphurous, where sodium and soluble chloride at the range of 11,000-34,000 ppm and 11,000-99,000 ppm, respectively, while sulfate varied from 2,070 to 18,8200 ppm. Next generation sequencing (454 pyrosequencing) was applied to determine the bacterial communities inhabiting each layer.

### **Conclusions**

Overall, *Pseudomonas* Genus was dominated in both samples with 46% and 39% for I and II, respectively. *Pseudomonas putida* was identified in each cross-section at 1cm with variable percentages. In addition, *P. veroniim*, *P. cannabina* and *Herbaspirillum rubrisubalbicans* were presented with less percentages. *Leptospirillum* spp. were detected at lower layers and at they were optimized between 15-20 cm while *Methylobacterium tardum* appeared at their maximum around 7-9 cm. Most of genera saline species of *Halobacteriaceae* family like *Halorhabdus* spp., *Halonotius* spp., *Halobaculum* spp., *Halomicrobium* spp. *Halorubrum* spp., *Halogranum* spp., *Haloplanus natans*, *Salinibacter* spp. and *Natronomonas* spp. were detected within 1-2 cm near to the salty crusty surface of Sabkah.

**FEMS-2445**

**Extreme environments**

**CONSTRUCTION OF PUC19 PLASMID METAGENOMIC LIBRARY FOR EXPLORING NOVEL PROTEASE ENZYMES FROM LAKE ACIGOL, A HYPERSALINE LAKE IN SOUTHERN, TURKEY**

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**Background**

Investigations of extreme environments are important for the study of evolution relationships, discovery of new species and various ecological relations among organisms. Besides, new metabolites and novel enzymes, metabolic pathways of organisms' can be discovered from extreme environments.

**Objectives**

In this study, metagenomics approach is used to isolate potential biotechnological novel enzymes from Lake Acıgöl. Especially we have focused on proteases which are ubiquitous enzymes and they have crucial roles in different industrial applications. Research area Lake Acıgöl is located between Afyon, Denizli and Burdur city boundaries in Aegean region, Turkey. It is a good example for extreme environments due to its high salinity (about 200g/L NaCl) which makes the lake suitable for halophilic microorganisms.

**Methods**

Bacterial population of Lake Acıgöl have already been identified and Lake bacterial population consist of 38.8% Uncultured Bacterium, 30.8% Firmucutes, 15.3% Bacterioidetes, 7.6% Gammaproteobacterium and 7.6% Deltaproteobacterium. Because of the high population of uncultured microorganisms Lake Acıgöl is a potential candidate for novel enzyme exploration. To isolate extremophilic protease enzymes from Lake Acıgöl sediment samples were collected from different part of the Lake and Metagenom (DNA) isolation was carried out by manually according to Zhou et. Al., 1999 [1].

**Conclusions**

Metagenom library was constructed by using *pUC19 plasmid*. Screening studies for possible protease enzymes will be carried out using 1% of skim milk LB agar plates and Salt Tolerance Screening Method according to Culligan et. Al., 2013 [2].

**FEMS-1862**

**Extreme environments**

**CUPRIAVIDUS METALLIDURANS CH34 PHYSIOLOGY AND INTERACTIONS WITH BASALT ON EARTH AND IN SPACE**

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**Background**

Microbe-mineral interactions have become of interest for space exploration as microbes can biomine useful elements from planetary regolith, which could serve as nutrients in a life support system.

**Objectives**

Therefore, this research aimed to assess the impact of space conditions on physiology of *Cupriavidus metallidurans* CH34 and identifying the molecular mechanisms behind microbe-mineral interactions on basalt, a lunar-type rock.

**Methods**

Survival of *C. metallidurans* CH34 was monitored after a 3-month period in mineral water with or without basalt by plate counts, flow cytometry, ICP-MS and microscopy. The effect of space conditions was studied via a flight experiment on board the Russian PHOTON-M4 capsule.

**Conclusions**

The results obtained from ground experiments showed that the cultivable fraction dropped to 10% indicating a transition to a more dormant state. In the presence of basalt, CH34 remained viable, attached and formed a biofilm. The space flight experiment indicated more viable cells compared to the ground experiment both in the absence and presence of basalt, indicating a positive effect of space flight on survival.



Additional physiological and molecular analyses are on-going to confirm these observations and to determine the molecular processes.

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**FEMS-1514**

**Extreme environments**

**MEMBRANE-BOUND AMYLOPULLULANASE IS ESSENTIAL FOR STARCH METABOLISM OF SULFOLOBUS ACIDOCALDARIUS DSM639**

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**Background**

The growth of *Sulfolobus* shows that these species prefer starch or long-chain maltooligosaccharides to maltose as a carbon source and have both  $\alpha$ -amylase and  $\alpha$ -glucosidase activities. The annotation of the genome sequence of *S. acidocaldarius* assume that the gene encoding amyolytic enzyme, Saci\_1162, within the putative maltose/maltodextrin ABC transporter operon (*Saci\_1160* to *Saci\_1166*) is involved in the degradation of starch or  $\alpha$ -linked polymers into small maltooligomers.

**Objectives**

A putative amyolytic enzyme, Apu (Saci\_1162), was characterized whether it is involved in the starch metabolism in *S. acidocaldarius*.

**Methods**

The physiological role of Apu in starch metabolism was investigated by the growth and starch degradation pattern of *apu* disruption mutant as well as biochemical properties of recombinant Apu.

**Conclusions**

The  $\Delta$ *apu* mutant lost the ability to grow in minimal medium in the presence of starch, and the amyolytic activity observed in the membrane fraction of the wild-type strain was not detected in the  $\Delta$ *apu* mutant when the cells were grown in YT medium. The purified membrane-bound Apu initially hydrolyzed starch, amylopectin, and pullulan into various sizes of maltooligosaccharides, and then produced glucose, maltose, and maltotriose in the end, indicating Apu is a typical endo-acting amylopullulanase which belongs to glycosyl hydrolase family 57. The maltose and maltotriose observed in the culture medium during the exponential and stationary phase growth indicates that Apu is the essential enzyme to initially hydrolyze the extracellular starch into small maltooligosaccharides to be transported into the cell.

**FEMS-0495**

**Extreme environments**

**MICROBIAL COMMUNITY PROFILE OF THE IRON-SULPHATE RICH WATERS OF RIO SUCIO (BRAULIO CARRILLO NATIONAL PARK, COSTA RICA)**

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**Background**

Río Sucio ('Dirty river') in Braulio Carrillo National Park (Costa Rica) is a river of volcanic origin that possesses a characteristic yellow color due to the presence of high amounts of mineral deposits from Irazu Volcano.

**Objectives**

To perform a physicochemical characterization of the Río Sucio water and to analyze the impact of these parameters on the microbial community that inhabits the river.

**Methods**

Physicochemical analysis was performed by ion-exchange chromatography (IC), inductively coupled plasma mass spectrometry (ICP-MS), scanning electron microscopy with electron dispersive spectrometer (SEM-EDS) and X-ray diffraction (XRD). Analysis of the microbial community was performed by analysis of the V5-V6 hypervariable regions of the 16S rRNA gene. PCR amplicons were sequenced on a Illumina MiSeq instrument and the sequences compared against the RDP database (Ribosomal Database project – <http://rdp.cme.msu.edu/>).

**Conclusions**

Physicochemical analysis revealed that Río Sucio is a slightly acidic river (pH 5.0) containing high concentrations of sulphate ( $502 \pm 29$  mg/L), calcium ( $96.6 \pm 2.2$  mg/L), magnesium ( $27.36 \pm 0.16$  mg/L), aluminium ( $17.1 \pm 1.9$  mg/L) and iron ( $5.20 \pm 0.11$  mg/L). The precipitated material in the bottom of the river was composed mainly of iron oxides, as indicated by SEM-EDS and XRD. Microbial diversity analysis showed that the most abundant organisms are closely related to sulfur, thiosulphate and iron oxidizing bacteria such as *Sulfuritalea* spp., *Ferrovum* spp., *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Ferrithrix* spp. These observations suggest that Río Sucio has an acid rock drainage environment where the chemical composition is biologically driven by sulfur and iron oxidizing bacteria.



## FEMS-2322

### Extreme environments

#### LIFE IN A MICROWAVE: EFFECTS OF MICROWAVE RADIATION ON THERMUS SP.

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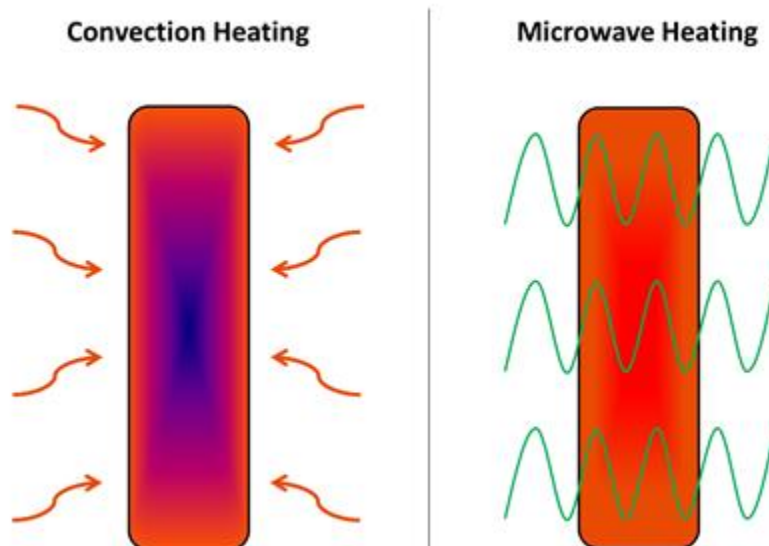
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#### Background

Biological systems are frequently exposed to microwave radiation. Many studies have investigated the influence of microwaves on these systems, but controversy over methods to distinguish between thermal and non-thermal microwave effects remains.



#### Objectives

To differentiate between non-thermal and thermal microwave effects on a physiology of a microorganism, a thermophilic bacterium was grown in a constant-temperature microwave or a convection oven. Comparing the growth properties of the thermophile in these conditions will reveal non-thermal microwave effects on cell growth and

physiology. Biophysical and biochemical analysis will demonstrate changes in morphology and chemical composition arising from microwave exposure.

### **Methods**

Cell growth was analyzed by optical density (OD) measurements (supported by independent quantitative DNA analysis), and cell morphologies were characterized using electron microscopy imaging (SEM, TEM), dynamic light scattering (DLS), and atomic force microscopy (AFM). AFM was also used to probe the biophysical characteristics of the cells, in conjunction with nano-infrared spectroscopy (Nano-IR). Attenuated total reflectance infrared spectroscopy (ATR-IR) and fatty acid methyl ester (FAMES) analysis were used to determine biochemical differences between cells grown in microwave and oven conditions.

### **Conclusions**

Thermophilic bacteria were grown in a synthetic microwave such that thermal effects and microwave effects were distinguishable. These data demonstrate that there are physiological differences between cells cultured in a dielectric field and a convection oven, and that microwaves induce non-thermal changes to the structure, physiology, and chemical composition of the organism.

**FEMS-1718**

**Extreme environments**

**DO THE PROTEINS INDUCED BY HIGH HYDROSTATIC PRESSURE IN FUNGI  
PLAY A ROLE IN TOLERANCE TO PRESSURE?**

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**Background**

Deep-sea is a fascinating environment, which inspite of the extreme conditions of pressure, temperature and nutrients, is home to many life-forms. In order to survive here, the organisms need to develop mechanisms to counter these conditions. Fungi, one of the most ubiquitous eukaryotic forms of life, have also been reported from deep-sea sediments. In order to survive, they undergo both morphological and biochemical adaptations. Induction of proteins is one such response elicited by fungi for survival.

**Objectives**

In our study to understand the mechanism of survival by fungi occurring in deep- sea conditions, we are looking for proteins expressed by fungi and their role in tolerance to high hydrostatic pressure using LCMS QToF.

**Methods**

To understand the mechanism of survival of fungi, in deep- sea conditions, we examine tryptic digests of proteins expressed under various hydrostatic pressure using LCMS QToF.

**Conclusions**

The results showed that some proteins were up-regulated whereas some were down-regulated at higher pressures. Some over-expressed proteins, probably playing a role in pressure tolerance were 30 KDa heat shock protein and heat shock protein SSC1 mitochondrial. Unfortunately, none of the up-regulated proteins could be considered as ones produced exclusively in response to high hydrostatic pressure as all are reported in response to other stresses also. Along with these proteins, there were several hypothetical proteins to which no function has been assigned so far. These could well prove to be the ones limited to pressure stress, which needs to be investigated further by either inactivating the proteins or using knock-down mutations.

**FEMS-2556**

**Extreme environments**

**POLYPHASIC CHARACTERIZATION OF A NEW EXTREMELY HALOPHILIC  
ARCHAEON ISOLATED FROM URMIA SALT LAKE IN IRAN**

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**Background**

Urmia Lake in the Northwest of Iran is the second largest hypersaline lake in the world which has been explored with great haloarchaeal diversity. Extreme halophilic Archaea are widely distributed in hypersaline habitats.

**Objectives**

During the course of biodiversity studies in Urmia Lake, several new extreme halophilic Archaea were isolated. Amongst them we chose strain DC8<sup>T</sup> for further characterization.

**Methods**

Strain DC8<sup>T</sup> has been characterized taxonomically using polyphasic approach. According to minimal standards we performed Molecular, biochemical, morphological and physiological tests for characterization.

**Conclusions**

16S rRNA gene sequencing showed that strain DC8<sup>T</sup> is a member of the family *Halobacteriaceae*; however, its similarity was as low as 90.1% and 89.3% to haloarchaeal taxa including *Halosimplex* and *Halobaculum* type species, respectively. The cells of strain DC8<sup>T</sup> were non-motile and pleomorphic and needs at least 2.5 M NaCl and 0.02 M MgCl<sub>2</sub> for growth. Optimal growth was at 4.0 M NaCl and 0.1 M MgCl<sub>2</sub>. The optimum pH and temperature for growth were pH 7.5 and 45°C. The G+C content of its DNA is 68.1 mol%. Polar lipid analyses revealed that strain DC8<sup>T</sup> contains phosphatidylglycerol, phosphatidylglycerol phosphate methyl ester and phosphatidylglycerol sulphate. The only quinone present was MK-8 (II-H<sub>2</sub>). The physiological, biochemical and phylogenetic differences between strain DC8<sup>T</sup> and other previously described genera of extremely halophilic archaea suggest that this



strain represents a novel species in a new genus within the family *Halobacteriaceae*, for which the name *Halositis urmiana* gen. nov., sp. nov. is proposed.

**FEMS-1534**

**Extreme environments**

**LIFE ON THE EDGE: PROTEOBACTERIA AND HALOARCHAEA DOMINATE THE MICROBIAL COMMUNITY OF AN EXTREMELY HIGH ALTITUDE LAKE, LAKE LLULLAILLACO (6170 M)**

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**Background**

Despite the crucial role of microorganisms in lake ecosystem function, they are typically poorly studied in high altitude ecosystems. In general, there are few reports of microbial diversity in lakes located at altitudes >5000 m. Lake Llullaillaco is a previously undescribed lake located at 6170 m on the Chilean side of the Llullaillaco volcano, the second highest volcano in the world (6739 m).

**Objectives**

The aim of this study was to describe the basic morphology of Lake Llullaillaco and the microbial diversity from water samples taken from the lake in February 2013.

**Methods**

Bacterial cultures were obtained using different culture media and microbial diversity was analysed by the pyrosequencing of 16S rRNA genes of Bacteria and Archaea. At the time of sampling, the lake had an area of 0.9 ha and maximum depth of 6.8 m. Water temperature ranged between 2 and 5°C and pH was 6.5. 24 bacterial isolates were obtained belonging to the Alpha-, Beta-, Gammaproteobacteria and Actinobacteria. Bacterial diversity was dominated by Proteobacteria (86.5%), Actinobacteria (11.9 %) and Bacteroidetes (1.4%), with Beta-, and Alphaproteobacteria being the most abundant taxa. Archaeal diversity was dominated by Euryarchaeota and the Halobacteriaceae, with a high contribution of unidentified sequences.

**Conclusions**

This study is the first description of microbial diversity in Lake Llullaillaco: diversity was low and characterized by a combination of phylotypes previously described from cold environments and a series of undescribed microorganisms.

Acknowledgements: Fondecyt Regular N°1140179, 1110953; Centre CeBiB FB 0001

**FEMS-1211**

**Extreme environments**

**MICROBIAL DIVERSITY IN ARMENIAN SALINE-ALKALINE SOILS AND SUBTERRANEAN SALT DEPOSITS ANALYZED BY MOLECULAR AND CULTURE-BASED METHODS**

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**Background**

One of the main directions of microbial ecology of extremophiles is the study of halophilic microorganisms' biocenoses, which are mainly found in saline lakes and other water systems as well as saline soils and salt mines. Microbial biodiversity of salt mines and saline-alkaline soils distributed on the territory of Armenia is still poor investigated.

**Objectives**

The aim of the present study was to investigate the microbial composition of saline-alkaline soils of the Ararat Plain and Avan subterranean salt deposits in Armenia.

**Methods**

For this study, clone-library construction of PCR-amplified 16S rRNA genes, DGGE-PCR fingerprinting and cultivation-dependent methods were used. 16S rRNA gene clone libraries and PCR-DGGE fingerprinting sequences were generated from total DNA extracts using universal archaeal and bacterial oligonucleotide primer sets.

**Conclusions**

Sequence analysis of bacterial and archaeal clone libraries and DGGE-PCR products from the samples indicated a dominance of *Firmicutes* and *Euryarchaeota* from the bacterial and archaeal domains, respectively. Several aerobic chemoorganotrophic endospore-forming bacteria were isolated from the salt samples and identified as representatives of the genus *Halobacillus*, *Piscibacillus*, *Virgibacillus* and *Streptomyces*. Five halophilic archaeal strains were isolated from salt stone samples. Four of the archaeal strains were most closely related to members of the genus *Haloarcula* (97-99 % similarity) and one strain most closely to the genus *Halarchaeum* (<97% similarity), indicating that Avan salt mine harbors a unique community of possible novel species.

The work was supported by the CPEA-2011/10081 grant from the Norwegian Cooperation Programme in Higher Education with Eurasia.

**FEMS-2444**

**Extreme environments**

**COPQ, A NOVEL FAMILY OF SMALL SECRETED PROTEINS WITH PUTATIVE RADICAL-SCAVENGING AND METAL-DETOXIFYING ACTIVITIES, ARE STRICTLY CONFINED TO THE GENERA RALSTONIA AND CUPRIAVIDUS**

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**Background**

The soil bacterium *Cupriavidus metallidurans* CH34 is tolerant to a wide range of metals (1). Whole-genome transcriptomics demonstrated a common response in this organism for metal and oxidative stress (2). Some of the highly induced genes are well conserved within 14 *C. metallidurans* strains (3) and encode short proteins (coined the COPQ family) that possess a distinctive signal peptide and up to ten repetitive motifs [GSRD]XXD[PV][YF]T[DE]G[ASG].

**Objectives**

Bioinformatic analysis of the COPQ family.

**Methods**

Transcriptomic procedures are in (2). Structural predictions were performed with I-TASSER and PHYRE-2. Sequence similarity analysis was done by iterative BLAST against the non-redundant protein sequence database (nrdb – version 2014/09/10). Motif detection was done using BioGrep against 2,616 bacterial proteomes (refpep – version 2014/01/09).

**Conclusions**

BLAST analyses and BioGrep motif searches against reference protein databases showed that the CopQ family of proteins is strictly confined to the highly related species of *Cupriavidus* and *Ralstonia*. COPQ proteins are predicted to form straight, slightly helical polypeptides, probably owing to the abundance of chain-disturbing and evenly spaced glycines and prolines. The aromatic rings of the Tyr and Phe residues at the center of the motifs are fully exposed allowing easy access to reactive oxygen species (ROS).

We propose that COPQ-family proteins have a radical-scavenging function shielding cells from oxidative attack and/or metal toxicity. Highly purified COPQ proteins in single or mixed fashion are to be tested for their anti-oxidant and metal-binding

properties.

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**FEMS-1706**

**Extreme environments**

**BIODEGRADATION OF AROMATIC HYDROCARBONS BY HALOPHILIC  
ARCHEAE ISOLATED FROM ALGERIAN SEBKHAS**

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**Background**

Actually, the biodegradation of hydrocarbons (specialy PAHs) has recieved great attention, however, such high and fluctuating salinity promotes the loss of cell wall integrity, protein denaturalization, and changes in osmotic pressure, the biological treatment of industrial hypersaline waste-waters and the bioremediation of polluted hypersaline environments are not possible with conventional microorganisms.

**Objectives**

The aim of our study is the identification of microorganisms that could answer these criteria for the biodegradation of extreme environments.

**Methods**

Three strains halophilic archaea *Haloarcula* sp.D21(AM982816), *Halovivax* sp.A21(AM982815) and *Natrialba* sp.C21(HG423210) were isolated from the salt lake close to Ain Salah, Algeria. These strains show good growth on extreme saline culture media (up to 25%NaCl) in the presence of diesel, naphthalene and pyrene as the sole carbon source. The strain *Natrialba* sp.C21 has a better growth on these media even at 35% of salt. However, the highest rate of biodegradation of these three hydrocarbons is labeled in the presence of extreme halophilic consortium containing the three strains. This biodegradation is marked by the high rate of growth of the strains measured by the optical density, the production of biosurfactans estimated by the emulsifying index and by the lowering of the surface tension of the culture media (below 40mN/m). In addition, the biodegradation of these hydrocarbons in the presence of the halophilic consortium is correlated with the rate of the concentration of NaCl and Mg<sup>2+</sup> in the medium.

**Conclusions**

Thus, our halophilic haloarchae strains, specialy *Natrialba* sp.C21, are potential candidates for the degradation of pollutants at high salt concentrations.

**FEMS-2118**

**Extreme environments**

## **MICROCYSTIN VARIANTS IDENTIFIED IN ARCTIC CYANOBACTERIA USING A NEW ANALYTICAL SCREENING METHOD**

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### **Background**

Cyanobacteria produce a large variety of toxic secondary metabolites. In the Arctic and Antarctic, many unusual variants of microcystins (MCs) -the most common type of cyanobacterial freshwater toxins- have been detected. These variants are generally difficult to detect by standard methods in background-rich samples.

### **Objectives**

Therefore, there is a need to develop a screening method that can be used for a large number of environmental samples.

### **Methods**

In this study, 25 cyanobacterial samples from diverse biotopes in Svalbard (Arctic) were analyzed for the presence of MC. In a preliminary ADDA-MC specific ELISA, 18 of 20 samples were tested positive, an unusually high percentage. In contrast, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) precursor-screening, newly designed to identify candidate MCs from the ADDA fragment ions, suggested the presence of MCs in nine of the 25 samples. In three of the latter, MC variants could be subsequently confirmed and identified by complete MS/MS analysis as variants of MC-RR and MC-LA, similar to those previously reported for polar habitats. In five of the same nine samples, a gene involved in toxin production -*mcyE*- was successfully amplified, validating the analytical data. The amplified gene sequences were 93-98% similar to *mcyE* genes of *Nostoc*, indicating that this genus could be responsible for MC production in these samples.

### **Conclusions**

The presence of the unusual variants in the polar regions could help to understand the evolution and ecological function of the toxins. Moreover, the toxicological risk of unusual toxins is not yet known and might be an undetected threat.





## **FEMS-2150**

### **Extreme environments**

#### **'EX-SITU' PRESERVATION AND CHARACTERIZATION OF ANTARCTIC CYANOBACTERIA IN THE BCCM/ULC COLLECTION**

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#### **Background**

The BCCM/ULC public collection of (sub)polar cyanobacteria is funded since 2011 by the Belgian Science Policy Office. An ISO9001 certificate was obtained for the public deposition and distribution of strains, as part of the multi-site certification for the BCCM consortium. BCCM/ULC is currently holding 160 public cyanobacterial strains and the catalogue is available on <http://bccm.belspo.be/catalogues/ulc-catalogue-search>.

#### **Objectives**

Continuous maintenance of living cultures, some of which are also cryopreserved, ensure the preservation and the possibility to rapidly deliver strains to clients for fundamental and applied research.

#### **Methods**

The main holding of the collection concerns (sub)polar strains isolated from different biotopes and representative of a large taxonomic diversity. The molecular characterization is underway, on the basis of 16S rRNA and ITS sequences, but also Multiple Locus Sequence Analysis and genome sequencing.

In addition, cyanobacteria are known to produce a range of secondary metabolites (e.g. alkaloids, cyclic and linear peptides, polyketides) with various bioactivities. The potential of the polar strains to produce cyanotoxins and other secondary metabolites is currently studied by ELISA, LC-MS and the detection of genes involved in their production.

#### **Conclusions**

Due to the geographic isolation and the strong environmental stressors of the habitat, the exploration of these metabolites in Antarctic cyanobacterial strains seems especially promising for biotechnology or biomedical applications.

**FEMS-2826**

**Extreme environments**

**THE CRENARCHAEON *IGNICOCCUS HOSPITALIS* IS A POLYEXTREMOPHILIC ORGANISM WITH UNUSUAL HIGH RADIATION TOLERANCE**

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**Background**

The hyperthermophilic crenarchaeon *Ignicoccus hospitalis* was isolated from deep-sea hydrothermal vents living there strictly anaerobic and chemolithoautotrophic. From an astrobiological point of view, hyperthermophilic organisms are promising candidates for early Earth inhabitants, when the absence of oxygen, occasional meteorite impacts followed by heated up oceans, and the missing UV-absorbing ozone layer enabled higher radiation dose of short wavelength to penetrate Earth's surface.

**Objectives**

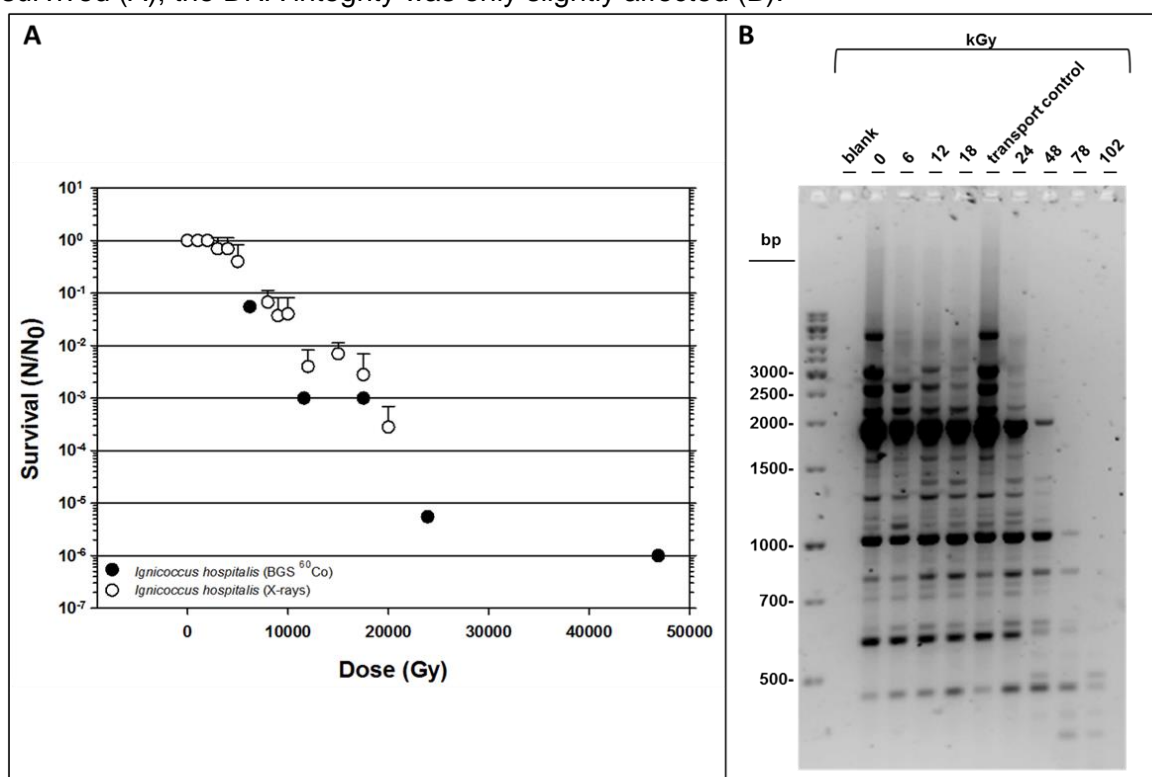
To ensure DNA integrity, *I. hospitalis* needs proper DNA repair mechanisms to cope with today's harsh environmental conditions in its natural habitat. These highly efficient DNA repair mechanisms might result in a tolerance to other yet untested harmful environmental impacts of astrobiological relevance. Therefore, *I. hospitalis*' radiation tolerance regarding ionizing and non-ionizing radiation has extensively been studied, and the DNA damage visualized on a molecular level. Future experiments will focus on repair kinetics and on the identification of proteins involved in DNA repair.

**Methods**

The survival after radiation exposure was determined by the most probable number technique (A), and PCR-based assays like RAPD (randomly amplified polymorphic DNA) analysis qualitatively confirmed changes in the genomic DNA after irradiation treatment (B).

## Conclusions

*I. hospitalis* cells showed a remarkable radiation tolerance although never exposed to it in its natural habitat. An exposure to 1000 J/m<sup>2</sup> monochromatic UV-C (254 nm) resulted in a reduction by 2-3 orders of magnitude which is in the same order as shown by *Deinococcus radiodurans*. An exposure to 24 kGy of ionizing radiation was survived (A); the DNA integrity was only slightly affected (B).



**A** Survival of *Ignicoccus hospitalis* after exposure to ionizing radiation. Cells were exposed to either  $\gamma$ -rays or X-rays. **B** RAPD profiles of genomic *I. hospitalis* DNA after ionizing radiation exposure.

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- Appl Environ Microbiol. 2004 Mar;70(3):1277-86. **Molecular diversity of new Thermococcales isolates from a single area of hydrothermal deep-sea vents as revealed by randomly amplified polymorphic DNA finger-printing and 16S rRNA gene sequence analysis.** Lepage E, Marguet E, Geslin C, Matte-Tailliez O, Zillig W, Forterre P, Tailliez P.



**FEMS-2721**

**Extreme environments**

**GENOMIC MODELLING OF S-LAYER PROTEIN IN EXTREMELY ACIDOPHILIC  
ARCHAEUM FERROPLASMA ACIDARMANUS FER1.**

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**Background**

*Ferroplasma acidarmanus* dominated the acid mine draining biofilm community in accordance with metagenomic metabolic modelling (Chen et al., 2012). One of the most impressive features of *F. acidarmanus* was its ability to survive at such an extreme proton gradient with only a cell membrane. The S-layer protects the cell against mechanical and osmotic stresses of extreme pH conditions. A rigid cell walls or S-layer were not documented neither in *F. acidarmanus* Fer1 nor *T. volcanium* as yet.

**Objectives**

Here we present an evidence for function of a gene coding for S-layer and the cellular localization of the protein in *F. acidarmanus* Fer1,

**Methods**

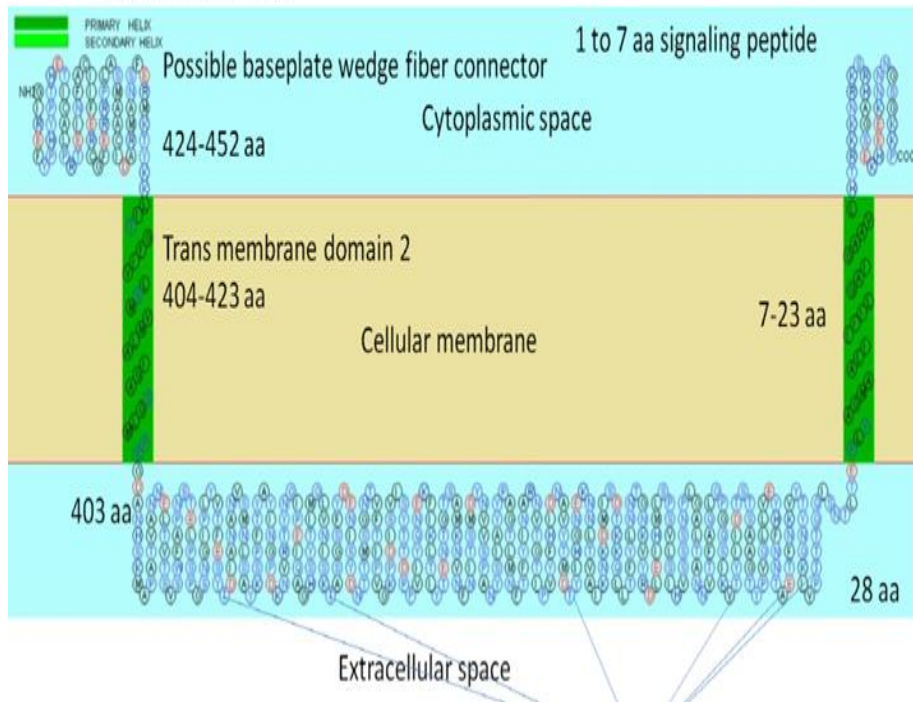
utilizing comparative tools including SignalP, TMHMM, SOSUI, Psort-B, Phobius, BLASTp, CDD, Pfam, Tigrfam, PDB, INTERPROSCAN, PrositeKEGG and Phylogeny.

**Conclusions**

The gene 638394352 was identified at 66-1422 (+) and coded for 452 amino acids with an isoelectric point of 10.08. The S-layer protein possessed a high content of acidic and hydrophobic amino acids. Protein played a structural role for the cell envelope and its biogenesis. We predicted two transmembrane domains of about 20 aa each anchored within the membrane while the majority of amino acids were located extracellular. The gene shared sequence homology with S-layer domain protein from *Sulfolobus islandicus*. The cellular localization model of 638394352 agreed with the cellular structure and localization of S-layer protein of orthologues *Sulfolobus*, which formed extracellular monomolecular crystalline mushroom-like

arrays of proteinaceous subunits on the cellular surface.

Figure 4. Model of cellular localization of protein product of OID 638394352 from *F. acidarmanus* fer 1 produced based on combined results of SOSUI, Phobius, SignalP, TMHMM and PSORT.



The peptide statistics, cellular localization, and protein homology, indicated that the gene was coding for S-layer domain..

**FEMS-1434**

**Extreme environments**

## **THERMAL ADAPTATION OF ESTERASES THROUGH THE EVOLUTION OF LOOP REGIONS**

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### **Background**

Temperature exerts multiple effects on the structure and function of enzymes which are still poorly understood at the molecular level. The adaptation of microorganisms to extreme temperatures includes the evolutionary development of biocatalysts with a high catalytic efficiency under these conditions. Nowadays, such extremophilic enzymes are used as valuable tools to study relationships between protein stability, dynamics, and function.

### **Objectives**

Here, we report on a comparative biochemical and molecular dynamics analysis of four homologous esterases belonging to the hormone sensitive lipase family which were isolated from bacteria living at temperatures ranging from 10°C to 70°C.

### **Methods**

The recombinant esterases showed highly similar substrate specificities. Furthermore, their optimal temperatures and thermostabilities resembled the temperatures of the respective bacterial habitats. Apparently, the thermal properties of these esterases were optimized to sustain the temperatures of their respective habitats. Therefore, we have used this set of enzymes for the analysis of molecular determinants beyond thermal adaptation.

### **Conclusions**

The structural analysis revealed significant differences between structures arising from surface exposed loop regions. The high flexibility of these structural domains was confirmed by molecular dynamics simulations performed at temperatures ranging from 15°C to 90°C. The results led us to propose four highly flexible loops (L2, L4, L10 and L12) distant from the active site as the structural regions most relevant for thermal stability. The link of such surface exposed loop-structures to thermal stability may indicate that natural thermal adaptation is achieved by tuning atomic interactions in “non-active site” loops without interfering with the catalytic function of an enzyme.



**FEMS-2271**

**Extreme environments**

**ANALYSES OF THE ANTIOXIDANT EFFECT OF COBALAMIN IN  
LEPTOSPIRILLUM GROUP II STRAIN CF-1 USING TRANSCRIPTOME AND  
PROTEOME APPROACHES**

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**Background**

Acid bioleaching environments are characterized by high levels of iron and heavy metals, which can increase the generation of reactive oxygen species and induce oxidative stress in microorganisms. Metaproteomic studies on *Leptospirillum* spp., a member of the bioleaching consortium, have shown that it contains high levels of cobalamin biosynthesis proteins. We have found that cobalamin exerts an antioxidant effect in *Leptospirillum* group II strains.

**Objectives**

The aim of this study was to clarify the possible mechanisms involved in cobalamin effect in *Leptospirillum* group II strain CF-1 through transcriptomic and proteomic approaches.

**Methods**

The sequencing and annotation of the genome were carried out using PacBio RS II technology and RAST software, respectively. Transcriptomic profile was obtained by RNA-seq using Illumina HiSeq 2500 sequencing. The mRNA abundance was calculated using Blast and expressed as RPKM (reads per kilobase per million) data. Proteomic assays were carried out using 1D-PAGE LC-MS/MS, and the LFQ intensities were normalized by MaxQuant software.

**Conclusions**

The genome analysis showed one circular chromosome with 2,709,324 bp and 3,070 coding sequences. Transcriptomic and proteomic analyses showed that cobalamin produces up-regulation of pathways involved in biosynthesis and repair of proteins, biosynthesis of iron sulfur clusters and amino acids, biofilm formation and energy metabolism proteins. Most of up-regulated pathways use S-adenosylmethionine or folate as cofactors which are products of cobalamin-dependent methionine and folate cycles. These data lead us to suggest that cobalamin could activate the central metabolism, improving the response to oxidative damage and restoring redox balance of the cell.

Acknowledgement: Fondecyt Grants 1120746, 1110203.

**FEMS-2280**

**Extreme environments**

**THE FE-S CLUSTER ASSEMBLY (ISC) SYSTEM IS FUNCTIONAL UNDER  
OXIDATIVE STRESS AND IRON STARVATION CONDITIONS IN THE  
ACIDOPHILIC BIOLEACHING BACTERIUM LEPTOSPIRILLUM GROUP II CF1**

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**Background**

The synthesis and assembly of Fe-S clusters is a highly complex and coordinated process in living cells. The ISC system is the housekeeping [Fe-S] cluster assembly system whereas the SUF system is functional under harsh environmental conditions such as oxidative stress and iron starvation. Members of *Leptospirillum* genus are acidophilic iron-oxidizing bacteria that belong to the consortium of microorganisms that participate in the bioleaching of ores and metal recovery. Interesting, despite to inhabit in a highly oxidizing environment with high concentrations of metals, these microorganisms have only one [Fe-S] cluster assembly system, ISC

**Objectives**

In this work we evaluated the functionality of the [Fe-S] cluster biogenesis system ISC of *Leptospirillum* Group II CF-1.

**Methods**

Bioinformatic analysis and RT-PCR experiments were used to characterize the genetic clusters that contain *isc* genes and to evaluate the co-transcription of genes. The expression level of key genes *iscR*, *iscS* and *hscB* was determined by quantitative RT-PCR analysis when cells were exposed to oxidative stress and iron starvation. The functionality of the ISC system was evaluated measuring the activity of cysteine desulfurase enzyme (IscS).

**Conclusions**

The results showed that *Leptospirillum* Group II CF-1 responds to oxidative stress and iron starvation by up-regulating the [Fe-S] cluster assembly *isc* genes and increasing the activity of IscS.

Acknowledgement: Fondecyt Grants 1120746, 1110203.

## **FEMS-2357**

### **Extreme environments**

#### **MOLECULAR INVESTIGATION OF THE RADIATION RESISTANT CYANOBACTERIUM ARTHROSPIRA SP. PCC8005**

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### **Background**

The cyanobacterium *Arthrospira* sp. PCC 8005 has been selected by the European Space Agency (ESA) for producing oxygen and food during future long-duration manned space missions, as part of the bio-regenerative life support system 'MELiSSA'. PCC 8005 must continue to produce oxygen and conserves high nutritive value while exposed to cosmic radiation in space.

### **Objectives**

The tolerance and the response of *Arthrospira* sp. PCC 8005 to ionizing radiation were investigated.

### **Methods**

Metabolically active planktonic cells of were exposed to Cobalt-60-generated gamma radiation and Helium and Iron particle radiation. The molecular response to radiation was investigated via photosynthesis, pigment, antioxidant, proteome and transcriptome analysis.

### **Conclusions**

The cells of PCC 8005 were able to survive doses of 6400 Gy of gamma, and 1000 and 2000 Gy of He and Fe particle radiation. During irradiation, cells switched quickly from an active growth state to a growth arrest mode, via a shut-down of photosynthesis and carbon fixation. Resources were rerouted to cellular protection and repair. Various antioxidant systems were activated, such as glutathione, to protect lipids, proteins and DNA. The cells activated also ssDNA repair systems and systems to remove damaged amino and nucleic acids from the cells. During recovery, the cells induced the *arh* genes, a new cluster of genes with unknown function. Finally, energy and metabolic pathways were restarted, and full recover of photosynthetic proliferation was obtained. These results show that *Arthrospira* sp.

PCC 8005 is a peculiar radiation resistant bacterium, useful for biotechnological applications, in space and on Earth.

**FEMS-2254**

**Extreme environments**

**MICROORGANISMS IMPACT ON GREENHOUSE GASES RECYCLING IN A HIGH ALTITUDE WETLAND (SALAR DE HUASCO, CHILE)**

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**Background**

Carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O) are three major greenhouse gases (GHG) which are naturally recycled mainly by microbiological mechanisms including respiration- CO<sub>2</sub> fixation, nitrification -denitrification and methanogenesis-methanotrophy. Microbial groups involved in GHG recycling have been detected through molecular surveys in high altitude wetlands such as Salar de Huasco. This site is a high altitude wetland characterized by its extreme physical-chemical environmental conditions (high radiation, daily temperature shifts, salinity gradients) and by the presence of novel groups within Bacteria and Archaea domains and complex microbial structures (mats) in freshwater and salt-saturated sites.

**Objectives**

The aim of this study was to determine atmospheric and dissolved GHG concentration in Salar de Huasco wetland and dry Altiplanic adjacent areas (3,800 - 4,000 masl) and the potential communities involved in its recycling during the austral dry season (November 2014).

**Methods**

GHG were measured in discrete samples from air, water and gas bubbles below microbial mats inhabiting fresh water areas of Salar de Huasco wetland by standard chromatography. Also molecular analyses of the communities were studied using massive sequencing (metagenomic and 16S rRNA barcode).

**Conclusions**

This study indicates that Salar de Huasco microbial communities process GHG very fast since despite notorious accumulation of CH<sub>4</sub> bubbles (over N<sub>2</sub>O) in cyanobacterial-microbial mats from fresh-water areas were found, the wetland was a net sink of GHG based on undersaturated values in the water and low ground air GHG levels compared with dry adjacent areas.

Acknowledgment: This work is part of the FONDECYT Project #1140356 and #1140179.

**FEMS-0515**

**Extreme environments**

## **HALOPHILIC FILAMENTOUS FUNGI FROM HYPERSALINE AND POLYHALINE ENVIRONMENTS**

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### **Background**

The existence of halophilic fungi in hypersaline environments has now been well established, negating the former belief that their presence was as simply ubiquitous fungi, or as being merely halotolerant. Several reports have indeed demonstrated the presence of these fungi in the Dead Sea and in solar salterns.

### **Objectives**

Although hypersaline environments have been recorded as the primary econiche of halophilic fungi, the objective herein, was to ascertain the presence of this extremophilic group of fungi in polyhaline environments of the estuary and mangroves, in addition to solar salterns, and to characterise their nature of halophily.

### **Methods**

Samples of water and sediment were obtained from these econiches and plated on to medium supplemented with high concentration of solar salt. The purified isolates so obtained were screened for their halotolerance levels, by growth on media containing increasing concentrations of salt.

### **Conclusions**

Halophilic fungi were indeed isolated from polyhaline environments, as well as from hypersaline solar salterns. Most of the halophilic fungi screened were found to possess moderate halophily; some of the isolates were true halophiles, having an essential requirement of salt for growth. It is thus shown that this group of extremophilic fungi may be found in hypersaline as well as non-hypersaline marine environments.



**FEMS-0948**

**Extreme environments**

**DEVELOPMENT OF BIOLOGICAL SOIL CRUST ON THE BARE SOIL MOUNDS AFTER SHRUB DEATH AND DECOMPOSITION IN A SEMI-ARID ECOSYSTEM**

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**Background**

**Background:** By the end of 2008, and following a prolonged drought period, a mass death of shrubs was observed in a semi-arid region of the Northern Negev Desert.

**Objectives**

**Objective:** This study followed the development of biological soil crusts (BSCs) on the soil mounds in the sites of the dead shrubs, spanning over a three year period, from the disappearance of the shrub skeletons until the mounds were flattened.

**Methods**

**Methods:** In addition to on-site physical measurements, BSC samples were collected and analyzed for their physical and biochemical properties (15-17 parameters) and their microbial community structure and compared to those of mature crusts.

**Conclusions**

**Conclusions:** Statistical analyses showed significant differences in the biochemical properties and the microbial community structure between the developing BSCs and the mature ones during five sampling campaigns. Interestingly, significant differences were observed also between the north-facing and the south-facing slopes of the mounds. The extent of these observed differences was linked to the sampling dates, implying an effect of the soil moisture. Significant differences persisted even after the mounds were totally flattened. This slow development of BSCs on the bare soil mounds after shrub death and decomposition in semi-arid ecosystems, may allow the establishment of new shrubs in these sites and the recovery of the affected ecosystem.



## FEMS-3026

### Extreme environments

#### GDGT LIPID COMPOSITION OF THE THERMOPHILIC ARCHAEA

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#### Background

The archaeal lipids differ significantly from the bacterial and eukaryotic analogs. The most studied lipids of thermophilic archaea are ether-bonded glycerides, resistant to usual lipid derivatization methods. Thus, characterization of archaeal lipid profiles is often not provided with the description of novel species.

#### Objectives

In our work, we have analyzed the composition of polar lipids, cellular fatty acids and the GDGT lipids of the several thermophilic archaea (*Thermoplasma* sp. DSM-1728<sup>T</sup>, *Desulfurococcus* sp. Z-1312<sup>T</sup>, and *Fervidicoccus* sp. Kam-940<sup>T</sup>), among them are several recently described species.

#### Methods

Polar lipids were studied by two-dimensional thin-layer chromatography [1,2], GDGT lipids were analyzed by GC-MS after iodination with subsequent reduction by Zn/CH<sub>3</sub>COOH [3]. Additionally, native GDGT lipids were analyzed by ESI-MS, and the polar groups of GDGT lipids were silylated and analyzed by GC-MS.

#### Conclusions

The polar groups of GDGT lipids of DSM-1728<sup>T</sup> contained phosphate, mannose, arabinose and gulose. This is confirmed by glycolipids and glycopospholipids revealed by TLC.

The GDGT lipids of *Thermoplasma acidophilum* DSM-1728<sup>T</sup> are dominated by GDGT4, which is confirmed by GC-MS analysis of core hydrocarbons. The polar lipid profiles of archaea studied are strikingly different, however, there are common glycopospholipids for the DSM-1728<sup>T</sup>, Z-1312<sup>T</sup>, and Kam-940<sup>T</sup> strains. The further study of lipids of these archaea by LC-MS will clarify their structures.

This work is supported by Russian Foundation for Basic Research (grant #13-04-40328) and by President of Russia (grant MK-4530.2015.4).

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**FEMS-2438**

**Extreme environments**

**USING A FUNCTION-BASED SCREEN TO IDENTIFY ACTIVE RUBISCOS FROM HYDROTHERMAL VENT ENVIRONMENTS: UNRAVELING REGULATION AND ACTIVATION OF A RUBISCO ENCODED ON A METAGENOMIC FRAGMENT**

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**Background**

RubisCO (Ribulose-1.5-bisphosphate Carboxylase/Oxygenase) is a key enzyme in the Calvin Benson Bassham cycle, which accounts for most of Earth's primary production. Although extensive work on RubisCO enzymes has been conducted in the last decades, we still know relatively little about the regulation and activation of RubisCO enzymes.

**Objectives**

We have developed a new activity-based screen that enables the identification of recombinant RubisCO active enzymes from metagenomic fosmid libraries. Using this screen we successfully harvested RubisCOs from a metagenomic fosmid library that was constructed from a deep-sea hydrothermal vent. After verifying the RubisCO activity, sequencing of one of the metagenomic fragments revealed a 35 kb DNA insert which encoded a RubisCO form I and form II.

**Methods**

We investigated the role of adjacent DNA regions on the RubisCO form I and form II regulation and activation by individually deleting each of the flanking genes and measuring the mutant's activity. Additional experiments with the genes' transcripts and band shift assays, to assess binding properties of the respective enzymes and genes, have furthered our understanding of how the RubisCO is regulated and likely activated.

**Conclusions**

This screen and this approach to work with the metagenomic fragments opens the door to directly studying RubisCOs from any environmental sample.

**FEMS-2266**

**Extreme environments**

**UV-RESISTANCE PROFILING OF A POLY-EXTREMOPHILIC  
GAMMAPROTEOBACTERIUM FROM HIGH-ALTITUDE ANDEAN LAKES**

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**Background**

High-Altitude Andean Lakes (HAAL) at the South American Andes are exposed to high UV irradiation and high content of toxic elements. A wide variety of microorganisms named “extremophiles” were found to inhabit these environments as *Acinetobacter* sp. Ver3<sup>1</sup> and it was chosen as model poly-extremophilic HAAL’s microbe as it displayed high resistance to UV-B.

**Objectives**

The aim of this work is to integrate diverse experimental approaches to profile the UV resistance mechanisms of Ver3, herein called as “UV-resistome”.

**Methods**

DSMZ collection strains were used for comparison with Ver3. Strains were exposed to different doses of UV-B, the resistance was determined by colony forming unit counting<sup>2</sup>. Survival, proteomic profiling and biofilm development after the exposure was evaluated by incubating cells under dark (DR) or light (PR) treatments. Genomic data were analyzed identifying components related with the “UV-resistome”.

**Conclusions**

Our results shown a superior resistance to UV-B radiation of Ver3 than control strains, in all cases the recovery was more efficient after PR, and the genome sequence supported the phenomenological observations. Ver3 highlighted a number of unique genes, such as a novel cryptochrome. An “UV-resistome” was defined, mainly genes related to UV-damage repair<sup>3,4,5</sup> on DNA and genes conferring an enhanced capacity for scavenging the reactive molecular species responsible for oxidative damage. The proteomic profiling of UV-challenged cells identified up-regulated proteins such as a specific cytoplasmic catalase, a putative regulator of quorum sensing, biofilm development and down-regulated proteins related to several energy-generating pathways. This is the first report on a genome from a polyextremophilic *Acinetobacter* strain.



**FEMS-1209**

**Extreme environments**

## **BACTERIAL DIVERSITY OF GEOTHERMAL SPRINGS IN NAGORNO-KARABAKH**

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### **Background**

The ability of microbes to thrive in high-temperature environments has prompted researchers to study these microorganisms to better understand their physiological and molecular adaptations and eventual utilization in various biotechnological applications. Natural geothermal springs, including terrestrial hot springs are one of the habitats of thermophilic microbes. On the territory of Nagorno-Karabakh many geothermal springs with different geotectonic origins and different physicochemical properties are found.

### **Objectives**

This research is focused on investigation of the bacterial community structure of previously unexplored geothermal springs of Nagorno-Karabakh, Karvachar (70°C) and Zuar (52°C).

### **Methods**

16S rRNA gene clone libraries were constructed from total community DNA using universal bacterial oligonucleotide primer sets. Sequences were used for phylogenetic assessment of the communities.

### **Conclusions**

Sequence analysis of clones indicated that clones obtained from Karvachar samples originated from phyla *Proteobacteria* (48.6%), *Cyanobacteria* (29.7%), *Bacteroidetes* (5.4%), *Chloroflexi* (5.4%), *Verrumicrobia* (2.7%) and *Planctomycetes* (2.7%) and clones obtained from Zuar samples originated from phyla *Proteobacteria* (42.3%), *Firmicutes* (19.2%), *Bacteroidetes* (15.4%), *Cyanobacteria* (3.8%), *Tenericutes* (3.8%) and yet unclassified phylotypes (15.4% for Zuar and 3% for Karvachar). The majority of the phylotypes detected in the gene libraries shared less than 95% sequence identity with their closest matches in GenBank, indicating a unique community structure of these geothermal springs. These thermal springs can represent a resource for novel thermophilic organisms and biotechnological tools.

The work was supported by the CPEA-2011/10081 grant from the Norwegian Cooperation Programme in Higher Education with Eurasia.





## **FEMS-1806**

### **Extreme environments**

#### **LIFE ON THE FROZEN CONTINENT: DIVERSITY OF RUBISCO, NIFH AND PUF<sub>LM</sub> GENES IN SOILS AROUND THE PRINCESS ELISABETH STATION, SØR RONDANE MOUNTAINS, ANTARCTICA.**

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#### **Background**

In Antarctica, photosynthesis by Cyanobacteria is generally thought to be the main primary source of organic carbon for complex microbial communities. Many cyanobacterial species are also able to fix nitrogen. Therefore, they can survive and prosper in almost every habitat, including Antarctica, Earth's most extreme continent. However, several studies of Antarctic microbial communities have shown that Cyanobacteria are not always highly abundant. We explored the hypothesis that other bacteria must take over their role and produce organic matter as well as fix nitrogen, in order to sustain the microbial community. Light is an abundant energy source during the Antarctic summer and some bacteria can use rhodopsin-type pigments to exploit this, whereas aerobic anoxygenic photosynthetic bacteria can use bacteriochlorophyll for photosynthesis.

#### **Objectives**

The presence and diversity of non-cyanobacterial prokaryotes that possess one or several of these properties was studied in terrestrial samples gathered in the proximity of the Belgian Princess Elisabeth Station (Sør Rondane Mountains, Queen Maud Land, East-Antarctica).

#### **Methods**

RuBisCO, *nifH* and *pufLM* genes were investigated by construction of PCR clone libraries and Illumina MiSeq sequencing.

#### **Conclusions**

Preliminary results indicate an extensive diversity of the genes coding for these processes in terrestrial Antarctica.

## **FEMS-2914**

### **Extreme environments**

#### **SOIL PROTEOMICS AND ATOMIC FORCE MICROSCOPY OF WATER-REPELLENT SOILS, A NEWLY RECOGNISED 'EXTREME' ENVIRONMENT**

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#### **Background**

Soil water repellency is a common phenomenon affecting the hydrological responses of many soil and land use types in different climates. This newly recognised 'extreme' environment leads to decreased water infiltration, reduced vegetation cover, fertiliser run off and soil erosion. The fundamental (biological) causes of soil repellency and its dynamic behaviour remain poorly understood.

#### **Objectives**

This study aimed to apply metaproteomic and high-resolution imaging approaches to model and predict switches between hydrophilic and hydrophobic soil surface responses.

#### **Methods**

Extreme, moderate and sub-critical water-repellent UK grassland soils, including Park Grass at Rothamsted Research, were sampled under wettable and repellent conditions. Soils were subjected to new extraction methods for determining the specific hydrophobic and the general metaproteomes and to Atomic Force Microscopy for determination of topological and adhesion properties.

#### **Conclusions**

Using our ultrahydrophobic extraction protocol, we identified novel ultra-hydrophobic microbial proteins, which likely play an important role in the development of soil water repellency. Such proteins could be extracted from moderate and extremely hydrophobic soils with medium-low soil moisture levels, but were absent in the comparable wettable soils. In control extractions, hydrophobic proteins were only extracted from wettable soil containing a positive control, demonstrating the specificity of our novel extraction method. Our newly developed metaproteomic method required only up to 0.5g of soil. Initial comparisons revealed differences in protein profiles, suggesting altered microbial ecophysiology in response to development of water repellency. Atomic force microscopy of repellent soils showed the nanoscale coverage of repellency on particles and aggregates by determining surface adhesion properties from force curves.

**FEMS-2575**

**Extreme environments**

**SPIRIBACTER SALINUS, A HALOPHILIC BACTERIUM DOMINANT ON  
HYPERHALINE HABITATS**

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**Background**

Metagenomic studies carried out in salterns located in Alicante and Huelva, Spain, revealed that in intermediate salinities ponds (13-21‰ of salts) inhabits a dominant bacterial group belonging to *Gammaproteobacteria*, related to *Alkalilimnicola*, *Arhodomonas* and *Nitrococcus*.

**Objectives**

On the basis of these metagenomic studies we designed different media and growth conditions in order to isolate this bacterium that have shown to be dominant at intermediate salinities. Subsequently, we studied the strategy of osmoregulation of this successful halophilic bacterium.

**Methods**

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**Conclusions**

We were able to isolate a microbe that has been cultured and described as *Spiribacter salinus*. Its complete genome has been sequenced and the recruitments against the available metagenomes of hypersaline aquatic habitats revealed its abundance in intermediate salinities, decreasing sharply at saturated and low salinities. *S. salinus* shows a simplified metabolic versatility, missing the chemolithotrophic and carbon fixation pathways.

We have combined physiological approaches and genomic analysis to derive a comprehensive picture of the molecular and cellular events that allow the adaptation of *S. salinus* to saline environments. A qualitative and quantitative assessment by natural abundance <sup>13</sup>C-NMR spectroscopy and high-performance liquid chromatography (HPLC) analysis, respectively, have shown that *S. salinus* is able to synthesize ectoine when is exposed to high salinity, showing an essentially linear relationship between ectoine content of the cells and the external salinity. We have also tested representatives of multiple transporter families that have been proved to sense and respond to osmotic stress, from ABC transporter as OpuA, BCCT transporter as OpuD and from the TRAP- transporter family, TeaABC.



**FEMS-2446**

**Extreme environments**

**MONITORING OF MICROBIAL CHANGES IN DRIPPING WATER OVER 5 YEARS  
IN HESHANG CAVE, HUBEI, CENTRAL CHINA**

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**Background**

Heshang Cave is a dissolution cave occurred in Cambrian dolomite. It is characterized by dark, oligotrophic, well-developed stalagmite and continuous dripping water throughout the year.

**Objectives**

Due to the great progress on the geochemical record and successful recover of microbial geolipids from stalagmite, it poses an urgent need to investigate microbial changes in terms of their relationship with environmental conditions which will help to decipher the microbial information in stalagmite.

**Methods**

Here we present a five year monitoring results about the variation of microbial communities in dripping waters in Heshang cave via 16S rRNA clone library construction and Biolog analysis of their carbon utilization. Meanwhile pH, temperature, conductivity, dripping rate, regional air temperature and precipitation were measured and collected.

**Conclusions**

Seasonal variations of bacterial communities were observed in composition, diversity and carbon utilization patterns. In autumn and winter bacterial community was dominated by Gammaproteobacteria, whereas in summer and spring Betaproteobacteria became dominant in water samples collected. Cluster and redundancy analysis indicated that regional air temperature strongly affect bacterial communities among the environmental factors investigated. Moreover the bacterial biodiversity was observed to increase with the temperature rising. These seasonal variation match well with the seasonal variation of microbial fatty acids in dripping waters in Heshang Cave which was also strongly affected by regional temperature. Our results show that microbial communities can response temperature change via multiple ways and the microbial variation recorded in stalagmite may be indicative of palaeo-temperature changes.

**FEMS-0567**

**Extreme environments**

## **VARIATIONS BETWEEN MICROBIAL COMMUNITIES IN DEEP SUBSURFACE BOOM CLAY PIEZOMETER INSTALLATIONS**

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### **Background**

The subsurface Boom Clay layer is investigated as a potential host rock for geological disposal of radioactive waste in Belgium. The HADES underground research facility (EIG Euridice c/o SCK•CEN), located at 230 m depth under the site of SCK•CEN (Mol, Belgium), provides access to this clay layer for *in situ* geological, geochemical and geomicrobiological testing.

### **Objectives**

In order to predict how microbiology will affect the biogeochemical processes in a disposal scenario, the resident microbial communities in the man-made structures within Boom Clay are being characterised.

### **Methods**

In this study, water samples were collected from Boom Clay via various existing piezometers (diverse in depth, orientation, location, age, materials used). The aim was to assess differences or shared features of the microbial communities residing in piezometer boreholes, and to correlate variations to geochemical analyses.

### **Conclusions**

Along the five piezometers, bacterial communities of the filters within one piezometer seem more similar to each other compared to those in other piezometers, despite a variety of filter materials or Boom Clay layers sampled within one piezometer. It shows that technical installations (such as piezometers) can introduce and promote local variations in the clay environment and the associated bioprocesses.

Further studies of other piezometers and of clay samples are needed, to pinpoint the source bacterial community underlying *in situ* enrichment, to unravel the mechanism that shapes such microbial community in different repository conditions and to outline the relevance of the (dominant) microbial classes in defining borehole water (and gas) chemistry.





## **FEMS-3115**

### **Host manipulation and bacterial survival**

#### **PHENOL-SOLUBLE MODULINS ALPHA ALTER THE CELL CYCLE OF EUKARYOTIC CELLS.**

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#### **Background**

*Staphylococcus aureus* (SA) is a Gram-positive bacterium responsible for a wide range of infections in humans and animals. We previously demonstrated that SA USA400 MW2 strain induces a G2/M phase delay in human HeLa cells. This phenomenon was associated with the accumulation of the cyclin-dependent kinase Cdk1/cdc2 and with the accumulation of unphosphorylated histone H3. Additionally we showed that the G2 phase was preferential for staphylococcal internalization and intracellular replication (1).

#### **Objectives**

The objectives of the study were

- to identify the active substances, which are responsible for the cell cycle alteration,
- to understand, how this alteration hijacks defense functions of the host cells.

#### **Methods**

Using size exclusion chromatography of MW2 supernatant, followed by mass spectroscopy analysis and FACS, we identified phenol-soluble modulin alpha (PSM $\alpha$ ) peptides as the likely candidates for cell cycle alteration.

#### **Conclusions**

Synthetic PSM $\alpha$ 1 and PSM $\alpha$ 3 caused a G2/M phase transition delay. The implication of PSM $\alpha$  in cell cycle alteration was confirmed by comparison of wild type LAC wt

strain with the isogenic mutant ( $LAC\Delta psma$ ), lacking the *psma* operon that encodes PSM $\alpha$ 1 to 4, for its internalization efficiency in HeLa cells. The decreased internalization rate of  $LAC\Delta psma$  suggested a role of PSM $\alpha$  in host cell invasion.

Furthermore, PSM $\alpha$ -induced G2/M-transition delay correlated with a decrease in the defensin genes expression suggesting a diminution of antibacterial functions of epithelial cells (2). Our results open new perspectives for the investigation of the mechanisms of the SA infection.

o 1 Alekseeva et al. Plos One 2013

o 2 Deplanche et al. FASEB. J 2015

**FEMS-1491**

**Host manipulation and bacterial survival**

**LACTOBACILLUS ACIDOPHILUS1 MAY PREVENT DIARRHEAL DISEASE BY VIBRIO CHOLERAЕ IN NEWBORN RABBITS**

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**Background**

Acute diarrheal disease-ADD is the second cause of death in the world in children under five years old and particularly in Latin America a good proportion is caused by *Vibrio cholerae*. The high incidence of ADD produced by *V. cholerae* is mainly due to antimicrobial resistance, being mainly to get control of cholera with probiotics our scientific interest.

**Objectives**

To assess the *in vitro* and *in vivo* capacity of *Lactobacillus acidophilus*1 to prevent diarrheal disease caused by *V. cholerae* 01OGAWA, in newborn rabbits. Our working hypothesis is to evaluate the use of lactic acid bacteria-LAB in the prevention and control of ADD.

**Methods**

Isolated and identified by biochemical and molecular tests *L. acidophilus*1, from 35 Colombian children stools. The amylolytic capacity and biomass formation by *L. acidophilus*1 were standardized previously. With *L. acidophilus*1, *in vitro* and *in vivo* antagonistic activities against the enteropathogen were performed and prevention of diarrheal disease was evaluated by *V. cholerae* in newborn rabbits.

**Conclusions**

The isolate was identified as *L. acidophilus*1, who in an inoculum of  $35 \times 10^6$  bacteria/ml was able to exert the most antagonistic *in vitro* effect on *V. cholerae*. After the *Kaplan–Meier* estimator, rabbits faced to the pathogen without receiving probiotic had lower survival probability of 0.25 compared to the group of animals challenged to the pathogen and simultaneously fed with probiotic whose probability of survival was 0.95. *L. acidophilus*1 is considered a probiotic microorganism, able to survive passage through gastrointestinal tract in an animal model and prevent intestinal colonization by *V. cholerae* in newborn rabbits.

**FEMS-2672**

**Host manipulation and bacterial survival**

## **HETEROLOGOUS EXPRESSION IN BUDDING YEAST OF DIVERSE BACTERIAL TRANSLOCATED EFFECTORS THAT TARGET COMMON CELLULAR COMPARTMENTS**

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### **Background**

Facultative and obligate intracellular pathogenic bacteria subvert host cell functions to create an intracellular environment for their replication and persistence. This is achieved by injection of bacterial proteins into the host cell cytoplasm via specialized type 3 or 4 secretion systems, thus reprogramming cellular pathways. Heterologous expression in the *Saccharomyces cerevisiae* yeast model is often used to understand the function of translocated effectors.

### **Objectives**

We report the expression in *S. cerevisiae* of over 30 candidate and cognate effectors from *Chlamydia trachomatis*, *Coxiella burnetii* and *Salmonella enterica* as N-terminal GFP fusions, aimed to study their subcellular localization as a means to gain insight in their function within eukaryotic cells.

### **Methods**

We present data on co-localization with fluorescent markers and the use of a battery of yeast mutants defective at specific membrane traffic stages to address differential cues recognized by these effectors.

### **Conclusions**

Most effectors showed a ubiquitous cytoplasmic distribution, either diffuse or concentrated in foci. Sometimes, as in *Coxiella* CaeA, these foci were consistent with accumulates of unfolded or aggregated protein. However, a few particular effectors displayed specific localizations. Notably, *Coxiella* AnkB was imported into the nucleus and associated to the nucleolus; *C. trachomatis* CT696, *C. burnetii* CBU-77 and *Salmonella* SteA specifically localized to cellular membranes. Particularly, *C. trachomatis* CT696 decorated the plasma membrane, *C. burnetii* CBU-77 localized at vacuolar membranes, and *Salmonella* SteA targeted simultaneously both

membranous systems. The utility of the yeast heterologous system to study the determinants for recognition by bacterial effectors of subcellular eukaryotic compartments will be discussed.

**FEMS-0694**

**Host manipulation and bacterial survival**

**C. CANIMORSUS AFFECTS COAGULATION BY REDUCING THE ACTIVITY OF VITAMIN K DEPENDENT CLOTTING FACTORS**

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**Background**

*Capnocytophaga canimorsus* is a Gram-negative bacterium belonging to the oral flora of dogs. The bacterium causes rare but severe infections in humans that have been bitten or licked. Patients frequently develop severe sepsis with disseminated intravascular coagulation (DIC).

**Objectives**

Considering that several bacteria interact with coagulation factors or platelets, as well as the frequency of bleeding abnormalities in *C. canimorsus* infection, we were interested if *C. canimorsus* interferes with coagulation.

**Methods**

We incubated normal pooled plasma (NPP) with *C. canimorsus* strain 5 (Cc5) and monitored thrombin generation by a calibrated automated thrombogram (CAT) assay. Additionally, clotting times of Cc5 treated NPP were measured. Factor deficient plasma was used to assess the activity of individual coagulation factors in Cc5 treated NPP.

**Conclusions**

Cc5 inhibited thrombin generation and clotting times of NPP incubated with Cc5 were significantly increased. However, pre-treatment of Cc5 with the irreversible serine protease inhibitor AEBSF completely abolished this increase.

We observed a specific impairment of the Vitamin K dependent (VKD) factors, FX, FIX, FII and FVII. We could also show that FX was cleaved by Cc5.

To conclude, our findings suggest a proteolytic mechanism by which Cc5 affects coagulation and which causes degradation of FX.

We are now interested in finding the cleavage site, to see if the other VKD clotting factors are equally degraded and to identify the bacterial protease which mediates the cleavage.

Inhibition of coagulation could promote bacterial dissemination and also aggravate DIC associated bleeding.



**FEMS-1087**

**Host manipulation and bacterial survival**

**V.VULNIFICUS DETECTED IN THE SPLEEN LEADS TO FATAL OUTCOME IN A MOUSE ORAL INFECTION MODEL.**

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**Background**

*Vibrio vulnificus* causes rapid disseminating septicemia by oral infection in infected individuals who have an underlying disease, especially chronic liver diseases. Although the elucidation of specific risk factors for *V. vulnificus* infection in patients with liver diseases is of urgent importance, no appropriate experimental animal model that mimics the liver diseases in this bacterial infection has been available so far.

**Objectives**

Discover the risk factors for *V. vulnificus* infection in liver diseases patients.

**Methods**

To discover these risk factors, we generated a liver disordered mouse by performing bile duct ligation (BDL). The BDL mice were infected with the *V. vulnificus* by orogastric route.

**Conclusions**

Hepatitis developed in the BDL mice, however this did not affect mortality in mice after orogastric administration of *V. vulnificus*, suggesting that the liver disorders caused by the BDL were not risk factors for *V. vulnificus* septicemia. When the dead and surviving mice were compared, *V. vulnificus* could be detected from the spleen only in the dead group. Furthermore, significantly higher numbers of *V. vulnificus* were detected from the intestines in the dead group than in the surviving group ( $p < 0.001$ ). These findings suggested that proliferation of the challenge inoculum in the intestine was needed for the oral infection with *V. vulnificus*, and that the elimination of *V. vulnificus* in the liver and/or spleen plays a critical role in survival of the host.



**FEMS-0534**

**Host manipulation and bacterial survival**

## **EFFECT OF HYPOBARIC HYPOXIA ON MICROBIAL HOMEOSTASIS AND PATHOLOGICAL CONSEQUENCES IN GUT**

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### **Background**

At high altitude (HA), hypobaric hypoxia is a hallmark of environmental stress that faced by millions of people like pilgrims, trekkers, scientists and military personnel and they generally suffer from a group of non-specific gastrointestinal complications like anorexia, dyspepsia, nausea, acidity, haematemesis, peptic ulcers, etc. These diseases are mostly related to the alteration of intestinal microbes and their related phenomenon.

### **Objectives**

This experiment was conducted to explore the relationship between the altered atmospheric pressure with the microbial composition, which is the major functional counterpart of GI tract.

### **Methods**

Albino mice were subjected to hypobaric atmospheric pressures (55kpa) in a chemostat for 15 days and after dissection intestinal luminal contents were collected. The population of indicator bacteria were monitored by selective culture based methods and DGGE. The expression of inflammatory markers of intestinal epithelium were evaluated by qPCR and western blot.

### **Conclusions**

The quantity of facultative anaerobes like *Escherichia coli*, other strict (*Bacteroidetes* sp. and *Lactobacillus* sp.) and obligate (*Clostridium perfringens*, *Peptostreptococcus* sp) anaerobes were increased in many folds after exposure to hypoxic environment. The increased level of bacteria and their endotoxins activated p38 MAP kinase and TLR4 pathways which are related to the over expression of inflammatory cytokines and mediators like IL23, IL17F, TNF  $\alpha$ , INOS, COX2 and HIF 1 $\alpha$ . As a result mucosal layers of intestinal wall was severely inflamed and perforated which also documented by SEM studies. These consequences are greatly related to the prognosis of different gastrointestinal ailments developed during hypobaric hypoxic stress at high altitude.



**FEMS-2071**

**Host manipulation and bacterial survival**

**IRON ACQUISITION MECHANISMS OF THE CYSTIC FIBROSIS (CF) PATHOGEN  
BURKHOLDERIA CENOCEPACIA AND THE CORRESPONDING HOST  
RESPONSE**

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**Background**

*B. cenocepacia*, is a highly antibiotic resistant CF pathogen which produces low molecular weight iron chelators known as siderophores, predominantly ornibactin, to sequester iron in the host. In response to bacterial and fungal infection, mammals produce lipocalins, some of which bind siderophores.

**Objectives**

To characterise ornibactin production by *B. cenocepacia* under different conditions and the resulting host lipocalin response to ferrated ornibactin and to investigate alternative iron acquisition mechanisms by this pathogen.

**Methods**

Ornibactin gene expression detected using real-time PCR and siderophore levels measured using the CAS assay. Utilisation of iron sources assessed using growth assays. Lipocalin 1 (LCN1) was expressed in *E. coli* using the Champion™ pET 100 vector system.

**Conclusions**

This study demonstrated that in iron-depleted cultures siderophore production by *B. cenocepacia* is significantly upregulated by 4 h ( $P < 0.001$ ) with maximal production by 5 h. Furthermore, *B. cenocepacia* can acquire iron from exogenous sources such as host iron binding proteins including ferritin and hemin, and from the *Aspergillus fumigatus* xenosiderophores, fusarinine C and triacetylfusarinine C demonstrating a multifaceted iron acquisition strategy. Host response investigations have determined that recombinant LCN1 binds ferric-ornibactin with high affinity and not unferrated ornibactin. Ongoing studies are examining cellular lipocalin responses and the utilisation of additional xenosiderophores. Understanding the iron acquisition strategies and host responses to this pathogen is warranted given the potential to prevent colonisation by this pathogen if iron acquisition can be compromised.

**FEMS-2507**

**Host manipulation and bacterial survival**

## **INFECTION OF CAMPYLOBACTER JEJUNI REDUCES CFTR MEDIATED CL- SECRETION IN T-84**

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### **Background**

*Campylobacter jejuni* is the common bacteria cause acute gastroenteritis in humans. It has been reported that the *C.jejuni*-infection was caused by very low infective dose, and it suggested that *C.jejuni* might have a resistance system against bacterial clearance system in intestinal tract. The mucus layer in the intestinal tract is the first defense system against bacterial infection, and the condition is well maintained by water movement. Cl<sup>-</sup> transport is closely associated with water efflux on intestine. Cystic fibrosis transmembrane conductance regulator (CFTR) is the major Cl<sup>-</sup> secretory pathway which is activated by cAMP. Dysfunction of CFTR causes dehydration of mucus and accumulation of bacteria in the intestine.

### **Objectives**

CFTR is tightly related with infection of pathogenic bacteria. However, it is not clear how CFTR related with the *C. jejuni*-infection. To elucidate the role of CFTR on *C. jejuni*-infection, we investigated the Cl<sup>-</sup> secretion in T-84 on infection of *C. jejuni*.

### **Methods**

Cl<sup>-</sup> secretion was measured by <sup>125</sup>I<sup>-</sup> efflux on T-84 cells. *C. jejuni*-infection did not change <sup>125</sup>I<sup>-</sup> effluxes in steady state. Next, we confirmed forskolin or prostaglandine E2, agonists of cAMP-dependent Cl<sup>-</sup> secretion, activated <sup>125</sup>I<sup>-</sup> effluxes, which inhibited by CFTR inhibitor.

### **Conclusions**

These indicated that *C. jejuni*-infection suppressed the activation of Cl<sup>-</sup> secretion dependent on CFTR. There were hypothesized that *C. jejuni*-infection effected on water movement and the mucus dehydration, which was related with bacterial clearance system in intestinal tract.



**FEMS-0569**

**Host manipulation and bacterial survival**

**A STRATEGY OF PATHOGEN STREPTOCOCCUS ESCAPE MAMMALIAN PEPTIDOGLYCAN RECOGNITION PROTEINS KILLING**

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**Background**

Our previous work showed that a new GI-type IVC secretion system location in the GI similar as previous described an 89K GI-like structure of *S. suis* named S4GIs is popular in the genus streptococcus.

**Objectives**

To reveal an unknown mechanism of a novel family of peptidyl-proly isomerases (PPIases) named SP1 from S4GIs interact with mammalian peptidoglycan recognition proteins (PGRPs).

**Methods**

In this study, we show that the function of killing bacteria of innate immunity proteins PGRPs could be blocked by protein SP1. We confirmed intracellular concentration of SP1 from streptococcus depends on secretion channel of S4G and increased expression of SP1 could be regulated by the concentration of PGRPs in environment using ELISA and qRT-PCR.

**Conclusions**

In conclusion, this study indicated a new escape way of pathogenicity Streptococcus protects against mammalian innate immunity proteins killing.

## FEMS-2781

### Imaging

## MASS SPECTROMETRY IMAGING OF ACTIVE CORRODING BIOFILMS IN 3 DIMENSIONS USING LASCA MSI

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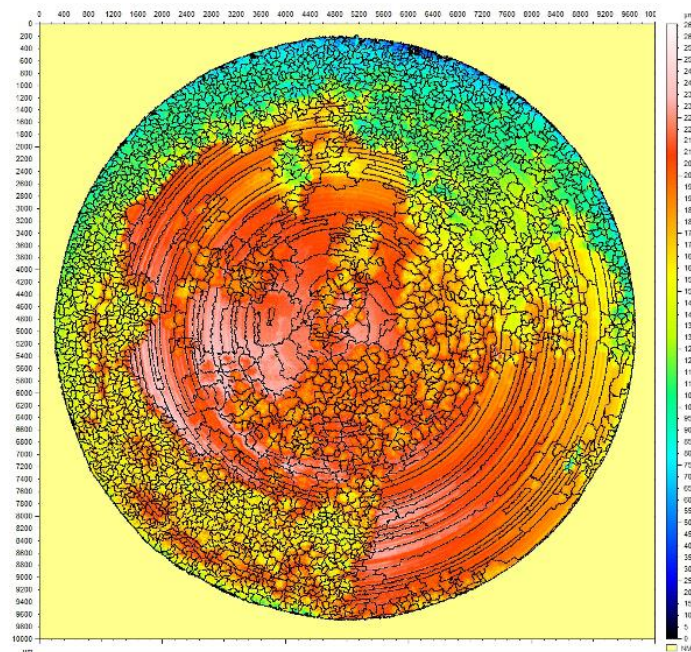
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### Background

Biofilms are highly diverse and complex multispecies microbial assemblages with characteristic spatial, i.e. three-dimensional organization. Elucidating function of a biofilm community would require a detailed characterization not only of the spatial distribution of the sessile organisms but also their respective metabolites.

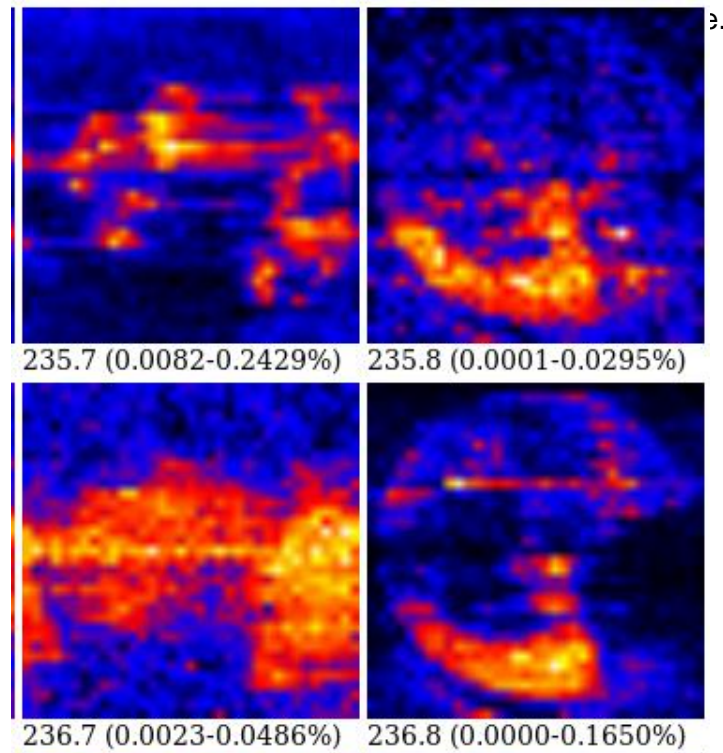
### Objectives

Mass spectrometry (MS) of homogenized biofilms (and other methods) have shown the importance of bacterial metabolites in the corrosion process. However, as seen in the profilometry map of a corroded coupon (Figure 1), the corrosion and the biofilm that causes it are not homogeneous. In order to gain a better understanding of the MIC process, we need to develop an instrument that can combine MS and spacial information.



### Methods

An ambient mass spectrometry imaging (MSI) system was developed specifically for imaging living biofilms on corroding lab or field samples. The Laser Ablation and Solvent Capture by Aerosol (LASCA) instrument uses an IR laser to collect a 200  $\mu\text{m}$  spot from the sample and analyzes it using a high resolution QToF. A single spot is analyzed in less than 10 seconds, allowing for thousands of spots to be analyzed and built into an ion image (Figure 2). The laser can be tuned to a specific



## Conclusions

We present a system that can create ion images of thick, uneven biofilms in 3 dimensions. We demonstrate the use of LASCA with the correlation of ion images to corrosion damage. Further, method to probe the penetration depth of biocides into a biofilm is presented.



## FEMS-2002

### Imaging

#### FLAVIN-BINDING FLUORESCENT PROTEINS - ADVANCED IN VIVO ANALYSIS OF BIOLOGICAL PROCESSES WITH HIGH SPATIO-TEMPORAL RESOLUTION

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#### Background

Genetically encoded fluorescent proteins (FPs), like GFP from the jellyfish *Aequorea victoria*, became one of the most variable and popular *in vivo* reporters in cell biology, microbiology and biomedicine. They can easily be detected by *in vivo* fluorescence techniques thus enabling the non-invasive analysis of complex cellular processes. However, beside the large size and relative slow maturation, a major drawback of GFP and its variants is their strict limitation to aerobic biological systems. This is primarily due to the fact that the autocatalytic synthesis of the chromophore depends on molecular oxygen.

#### Objectives

To conquer these limitations, we developed a new class of small cyan-green fluorescent proteins which can be used in microbes and mammalian cells under aerobic, oxygen-limited as well as anaerobic conditions. These FPs carry flavin mononucleotide (FMN) as chromophore and are thus termed FMN-binding fluorescent proteins (FbFPs).

#### Methods

To evaluate the applicability of FbFPs, the photophysical properties including the fluorescence spectra and brightness have been characterized and further improved. Furthermore, *in vivo* studies demonstrated their broad usability as reporter in (facultative) anaerobic bacteria and for bioprocess engineering. In addition, FbFPs were also be used to generate novel genetically encoded biosensors allowing the ratiometric intracellular monitoring of essential environmental parameters including molecular oxygen and pH.

#### Conclusions

The unique properties of FbFPs render them particularly valuable as *in vivo* tools for fluorescence imaging, quantitative *in vivo* analysis of gene regulation, protein

localization and bio-sensing.

**FEMS-2743**

**Imaging**

**TOWARDS STRUCTURE DETERMINATION OF MACROMOLECULAR  
COMPLEXES WITHIN THEIR NATIVE ENVIRONMENT**

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**Background**

Structures of macromolecular complexes are traditionally solved by X-ray crystallography, requiring crystals and bright X-ray sources to obtain resolutions at which side-chain densities can be seen. Last year, a revolution has taken place in the field of single-particle cryo-electron microscopy (EM), resulting in numerous high-resolution un-crystallisable structures. These breakthroughs have been made possible by the conjunction of a new generation of microscopes, detectors, and software.

In single-particle EM, thousands of noisy images of individual biomolecules are computationally combined into one 3D model. Best results are obtained when the sample of interest has been extensively purified and stabilized. During these steps, the biological context disappears, interacting partners dissociate, and unforeseen conformations could be induced.

**Objectives**

Ideally, single-particle like methods could be applied to biomolecules within their native environment. Unfortunately, almost all eukaryotic cells are too thick to be imaged by EM.

Our lab has set the long term goal to derive structures from biomolecules within their cellular environment. We label complexes with fluorescent tags to localize regions of interest (ROI) within vitrified cells. The coordinates of these ROIs are, together with the sample, transferred under cryogenic conditions into a dual-beam FIB/SEM system to trim the ROIs into ca 150nm thin lamella. Finally, the trimmed sample is cryogenically transferred to a high-end transmission EM for subsequent imaging at different orientations.

**Methods**

In here, we will report on the first results obtained with our cryo-correlative workflow on mycobacteria.

**Conclusions**

We discuss challenges and opportunities, the latter including developments such as phase plates and super-resolution microscopy.

**FEMS-1938**

**Imaging**

**AUTOMATED SEGMENTATION OF INDIVIDUAL CELLS AT PHASE CONTRAST MICROSCOPY IMAGES**

J. Soukup<sup>1</sup>

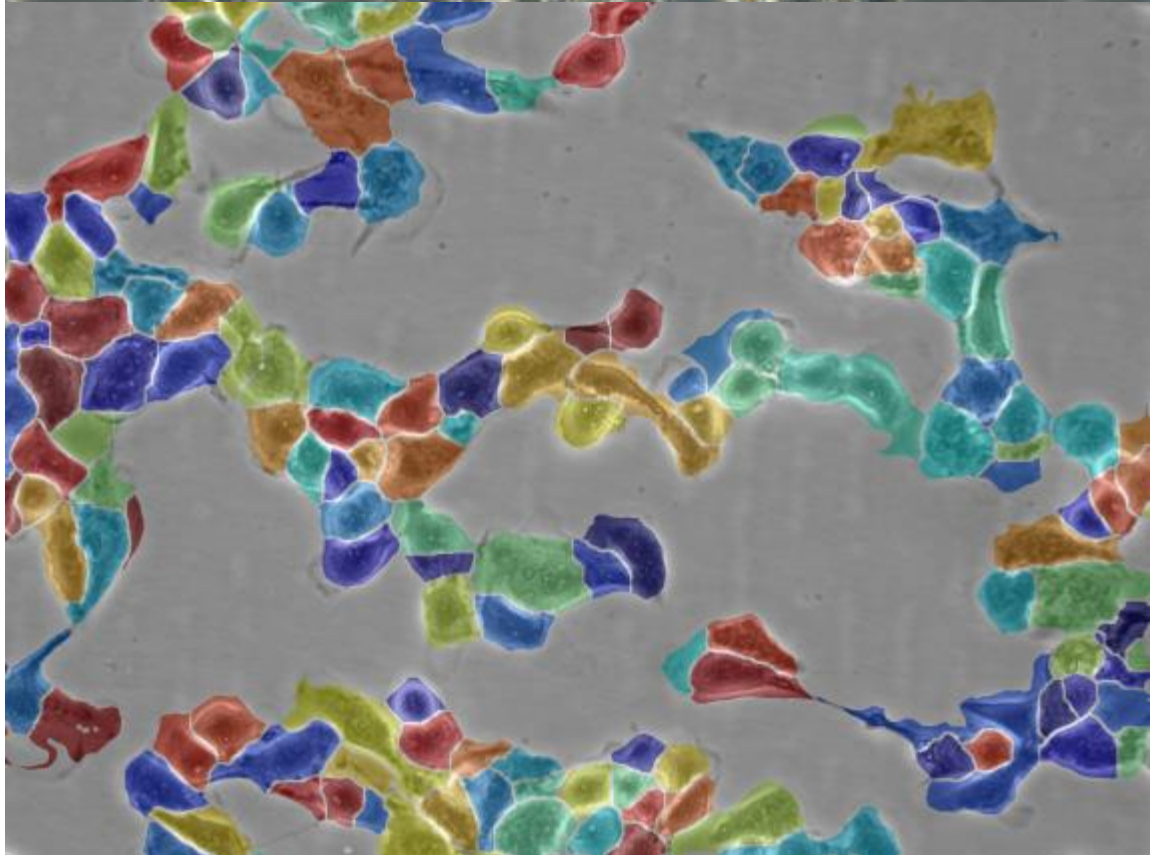
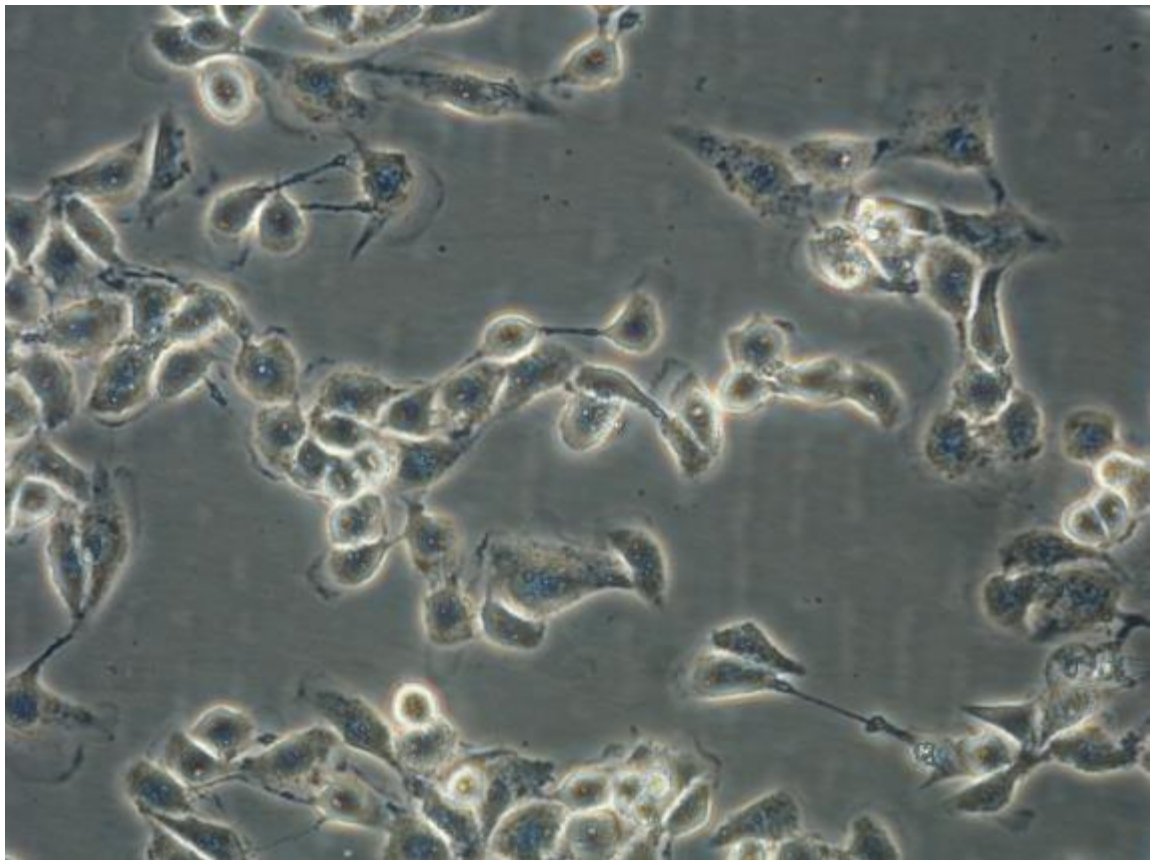
<sup>1</sup>*Laboratory of signal and image processing, ICS FFPW USB, Nové Hradý, Czech Republic*

**Background**

Nowadays, computer processing of microscopic images is becoming more common as a part of research in the field of cell science, replacing slow and time-consuming manual processing by human experts. A challenging task is for example to process images of live mammalian cancer cells from a phase contrast microscope.

**Objectives**

When processing the time-lapse images of live cells, the main goal is to describe the movement and behaviour of individual cells. There are many methods available in the cases when individual cells are separated from each other, However, separating individual cells from colonies or even from cell monolayers is much more difficult.



## **Methods**

We developed a novel algorithm for segmentation of individual cells. First part separate the cells from the background and it is based on the differences in time between consecutive images and a combination of sophisticated thresholding, blurring, and morphological operations. It is fast and precise. The second part of our algorithm separates individual cells in the clusters. It uses the halos between cells (thresholding and modified skeletonization) and fills the missing parts by connecting the hanging branches of the skeleton via Dijkstra algorithm.

## **Conclusions**

We tested the algorithm on images of four cell types acquired by two different microscopes, evaluated the precision of segmentation against manual segmentation performed by a human operator. We created the software which implements our segmentation method. We added the possibility to modify the resulting segmentation. User can modify the result by merging or splitting the cell regions that was found by out algorithm.

**FEMS-1563**

**Imaging**

## **STRUCTURAL STUDIES OF LEGIONELLA EFFECTORS**

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### **Background**

*Legionella pneumophila* is a Gram-negative bacterium and is the causative agent of Legionnaires' disease. The Dot/Icm type IV secretion system (T4SS) is one of the key virulence factors of *L. Pneumophila*. By transferring effector proteins into the host cell, the bacterium is able to replicate inside human alveolar macrophages by rapidly altering the normal host endocytic pathway after uptake. Currently, about 300 Icm/Dot dependent effectors have been identified in *L. pneumophila*; however, the biochemical functions for most of these proteins remain unknown.

### **Objectives**

Lpg1496 is an effector protein containing a conserved sequence of the SidE family in its C-terminal region. The SidE family members are present early during intracellular replication and localize to the cytoplasmic face of the replicative phagosome upon translocation. No information on other regions of lpg1496 has been available.

### **Methods**

We are exploring the three-dimensional structure of lpg1496 to probe for possible biochemical functions using structural similarity to proteins or domains of known function.

### **Conclusions**

Here, we identified two novel regions of sequence similarity in lpg1496 and showed that they form independently folded domains. Moreover, we have determined high-resolution crystal structures of these domains. Each domain consists of two  $\alpha$ -helices flanked by four  $\beta$ -strands on one side and four  $\beta$ -strands on the other side. Furthermore, we obtained the structure of the conserved C-terminal domain that shows structural similarity to HD domains and possesses phosphodiesterase activity. These studies will lead to a better understanding of the functional role of lpg1496 in *Legionella pneumophila*, and may contribute to the development of novel therapeutic treatments for Legionnaires' disease.



**FEMS-0845**

**Insect-microbes interactions**

**NOVEL BACILLUS THURINGIENSIS ISOLATES WITH LARVICIDAL ACTIVITY AGAINST THE MOSQUITO VECTOR, CULEX PIPIENS**

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**Background**

The outbreak of mosquito vectors, the incidence of mosquito-borne diseases and the resistance of mosquitoes to conventional pesticides have recently caused a panic to the official authorities in the endemic countries. The bacterium *Bacillus thuringiensis* (*Bt*), a safe eco-friendly entomopathogenic biocontrol agent, is widely used to complement the chemical control in integrated mosquito control measures.

**Objectives**

This study was conducted to identify native mosquitocidal *Bt* isolates to be utilized in the battle against insecticides-resistant mosquito populations.

**Methods**

*Bt* isolates have been recovered from different environmental samples collected from different locations throughout Saudi Arabia. Their mosquitocidal activities were tested against *Culex pipiens* 3<sup>rd</sup> instar larvae compared to that of the reference *B. t. israelensis* (*Bti*-H14) strain. Three isolates were almost as active as *Bti*-H14, showing LC<sub>50</sub> of 4.2-4.8 µg/ml. Seven isolates showed 1.6-5.4 times more toxic than that of *Bti*-H14 (LC<sub>50</sub> of 0.91-3.03 v 4.88 µg/ml). Histopathological examination of the *Bt*-63 isolate-treated larvae showed cellular and subcellular alternations in midgut epithelia similar to that caused by *Bti*-H14.

**Conclusions**

Data of this study showed that spore-crystal mixtures of 7 native locally isolated *Bt* were higher mosquito larvicidal compared to that of *Bti*-H14. Molecular characterization of these potentially active isolates and their toxic parasporal protein crystals are currently being investigated. This study may lead to the identification of mosquitocidal *Bt* isolate(s) that could contribute to the battle against mosquito

vectors.

**FEMS-2930**

**Insect-microbes interactions**

## **EFFECT OF GUT MICROBES ON THE EFFICACY OF BT MAIZE AGAINST LEPIDOPTERAN STEMBORERS**

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<sup>2</sup>*Zoology, North-West University, Potchefstroom, South Africa*

### **Background**

The evolution of resistance in agricultural pests to Cry proteins threatens the sustainable use of genetically modified Bt crops. The specific mechanisms of resistance are not well understood. According to the gut microbiota theory, extensive cell lysis caused by Cry proteins provide gut microbes access to the hemocoel where they germinate and reproduce, causing septicemia and death of the host.

### **Objectives**

The main objective of this study was to determine whether microbes present in the mid-gut of *Busseola fusca* influence the efficacy of Cry 1Ab proteins.

### **Methods**

Larvae were collected from maize fields and dissected to excise the mid-gut. Mid-gut contents (intestinal bacteria) were enumerated on general media. Different morphological types were selected to test the antibiotic susceptibility of the bacteria. The most effective bacteriostatic and bactericidal antibiotics were used and the morphological types were exposed to different concentrations of these to visualise the effects of the antibiotics. This consisted of growth curve studies on all the selected bacteria. A mixture of ciprofloxacin, ampicillin and doxycycline (500 µg/ml) was incorporated into an artificial diet. Stemborer larvae were allowed to feed on this for 7 days. These larvae were then placed on Bt maize (MON810) plant material expressing Cry proteins. Larvae actively fed on the plant material.

### **Conclusions**

Results suggest that by placing antibiotic reared larvae on a Bt plant, the absence of the mid-gut microbes contributed to larval survival on Bt maize.

**FEMS-3143**

**Insect-microbes interactions**

**GUT MICROBES OF BUSSEOLA FUSCA (LEPIDOPTERA: NOCTUIDAE)**

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**Background**

Genetically modified maize plants are engineered to express insecticidal toxins derived from the bacterium *Bacillus thuringiensis*. However, field-evolved resistance of African maize stemborers (*Busseola fusca*) against Bt-maize has developed and spread throughout South Africa. Studies suggested (1) that gut bacteria are required for *B. thuringiensis*-induced mortality in most Lepidoptera species and (2) that the toxicity of *B. thuringiensis* depends on microbial community interactions within the gut.

**Objectives**

This study aimed to identify the diversity of gut-associated bacteria by both culture-dependent and culture-independent approaches.

**Methods**

A total of 133 bacterial strains were isolated from the midgut of *B. fusca* larvae collected from 30 different sites. Molecular phylogenetic analyses of 16S rRNA gene sequences revealed bacteria affiliated to *Proteobacteria*, *Actinobacteria*, and *Firmicutes*. Taxonomic distribution of these sequences placed the isolates into 20 different genera. The majority of bacteria identified were belongs to the genera *Enterococcus*, *Klebsiella*, and *Bacillus*. Culture-independent methods involved the denaturing gradient gel electrophoresis fingerprinting (DGGE), and sequence analyses of a 16S rRNA gene from the excised band supported culture recovery results. However, additional bacterial taxa not determined via culture recovery were revealed using this methodology and included members of the genera *Chryseobacterium*, *Lactobacillus*, *Lactococcus*, and *Spiroplasma*.

**Conclusions**

Some sequences represent hitherto uncharacterized novel organisms that may have not been characterised yet. The *Busseola fusca* gut represents an intriguing and unexplored niche for analyzing microbial ecology, which will provide opportunities for research involving the impact of diverse and dynamic microbial communities on developing resistance against Bt-maize.

**FEMS-2557**

**Insect-microbes interactions**

**PRESENCE OF WOLBACHIA SP. IN DIFFERENT POPULATIONS OF CTENOCEPHALIDES FELIS: DEMOGRAPHIC HISTORY OR A WOLBACHIA SELECTIVE SWEEP?**

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**Background**

The cat flea, *Ctenocephalides felis*, is the most common flea species found on cats and dogs worldwide parasitizing a wide range of hosts. It has long been recognized that many arthropods carry passenger micro-organisms. *Wolbachia* alone infects in excess of 20% of insect species at any point in time. *Wolbachia*-infected species have been found to harbour lower levels of mtDNA diversity in closely related uninfected species.

**Objectives**

At the present work we carried out a molecular and phylogeographical study of *Ctenocephalides felis* and *C. canis* from different geographical regions (Spain, South Africa and Iran). Furthermore, the presence of *Wolbachia* sp. and its influence in these populations has been tested.

**Methods**

Molecular studies were based on the amplification and sequentiation of ribosomal DNA (Internal Transcribed Spacer 2, and 18S gene) and mitochondrial DNA (*cytochrome oxidase c-1* gene).

**Conclusions**

The presence of *Wolbachia* sp. was detected in the majority of fleas from different localities. The ribosomal data showed a great homology between all the populations of *C. felis* regardless the geographical origin but significative differences respect to *C. canis*. Nevertheless, *cytochrome oxidase c-1* sequences revealed significative differences in the population of *C. felis* from South Africa, appearing these sequences with high homology with that of *C. canis*. The endosymbiont *Wolbachia* could be responsible for selective sweeps on mtDNA variability within species. We suggest an introgression of mtDNA and *Wolbachia* between *C. felis* and *C. canis*.



**FEMS-1445**

**Insect-microbes interactions**

## **ANALYSIS OF THE IMPACT OF IRRADIATION ON SODALIS IN THE TSETSE FLY *GLOSSINA MORSITANS MORSITANS***

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### **Background**

Tsetse flies are the sole cyclical vectors of the African trypanosomosis that causes sleeping sickness in humans and nagana in animals. *Glossina* species have established symbiotic relationships with three bacterial species: *Wigglesworthia glossinidia*, *Sodalis glossinidius*, and *Wolbachia pipientis*, mainly known as a reproductive symbiont. A salivary gland hypertrophy virus that affects reproduction of infected flies has been reported. Vector control remains the most effective approach for sustainable management of African trypanosomosis. The sterile insect technique (SIT) has proven to be such an effective method and it is based on the mass production of the targeted insect, the sterilization by irradiation, and the sequential release of sterile males.

### **Objectives**

Such releases in endemic areas might increase the disease incidence before achieving eradication. Therefore, the development of symbiont-based strategies to produce tsetse strains refractory to trypanosome infection would be ideal for SIT programmes. One approach which is currently under consideration is to modify *Sodalis* to produce anti-trypanosome factor(s) in the released sterile males.

### **Methods**

We investigated the impact of irradiation on the establishment of *Sodalis* in the tsetse fly *Glossina morsitans morsitans*

### **Conclusions**

The results indicate that irradiating 5-7 days old male flies with 110 Gy does not increase the mutation rate, as assessed in 15 genes, but it does have a negative impact on the replication rate of *Sodalis* and the salivary gland hypertrophy virus. In contrast the irradiation treatment increased the prevalence of *Wolbachia*. These data are discussed in the frame of combining a symbiont-based and SIT-based control approaches.

**FEMS-0933**

**Insect-microbes interactions**

## **QUALITATIVE AND QUANTITATIVE ANALYSIS OF MICROBIAL DIVERSITY BY PYROSEQUENCING IN TETRANYCHUS URTICAE**

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<sup>2</sup>Department of Agricultural Biotechnology, Seoul National University, Seoul, Korea

### **Background**

Because of its extreme polyphagous nature, *Tetranychus urticae* is a good model organism to study the rapid adaptation mechanisms to various kinds of host plant toxins and acaricides. The symbiotic microorganism has been known as a pivotal factor in such chemical adaptation of many invertebrates. In *T. urticae*, several symbiotic bacteria (*Wolbachia*, *Cardinium*, etc) have been identified but research has been limited to the elucidation of their functional roles in cytoplasmic incompatibility.

### **Objectives**

Analysis of overall symbiotic bacterial composition in *T. urticae* is necessary as a cornerstone for the integrative study on its host adaptation and acaricide resistance development.

### **Methods**

16S rRNA pyrosequencing was conducted for nine *T. urticae* populations with different acaricide resistance properties (7 green types and 2 red types) with GS Junior Sequencing system (Roche, Branford, CT, USA) and data analysis was performed by CLcommunity program (Chunlab Inc., Seoul, Korea).

### **Conclusions**

The operational taxonomic unit by CD-HIT and TBC methods were estimated as  $85.3 \pm 22$  and  $737 \pm 220$  in average, respectively. Most bacterial species (>99%) were categorized in the order Rickettsiales mainly composed of *Wolbachia* spp. and *Rickettsia* spp. except for the acaricide-susceptible UD strain. The UD strain was composed of *Wolbachia* spp. (43.4%) and *Flavobacterium* (53.6%). Green- and red-type mites were grouped into different clades in the cluster analysis using the quantitative and qualitative traits, suggesting that the microbial diversity might be related with the phenotypic characters of body color. Further analysis would be



necessary to elucidate the functional roles of endosymbiont bacteria in regulating the intrinsic physiology of *T. urticae*.

**FEMS-0742**

**Insect-microbes interactions**

**ANALYSIS OF YEAST-LIKE SYMBIOTE DIVERSITY IN THE BROWN PLANTHOPPER (BPH), NILAPARVATA LUGENS STÅL, USING A NOVEL NESTED PCR-DGGE PROTOCOL**

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**Background**

Yeast-like symbiotes (YLS) are endosymbionts that are intimately associated with the growth, development, reproduction of their host, the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). However, it is unclear how many species of YLS are found within *N. lugens* and how they are related to each other.

**Objectives**

In order to overcome the difficulty in detecting low numbers of YLS in BPH or culturing the YLS *in vitro*, in this study, we developed a novel strategy, nested PCR–DGGE approach, which was used to analyze the diversity of YLS.

**Methods**

The nested PCR protocol was developed as follows: firstly, the 18S rDNA gene and 5.8S-ITS gene were amplified using fungal universal primers. Subsequently, these products were used as a template in a second PCR with primers ITS1GC-ITS2, ITS1FGC-ITS2 and NFGC-NR, which was suitable for DGGE. Using this highly specific molecular approach, we found several previously detected fungi: *Noda*, *Pichia guilliermondii*, *Candida* sp., and some previously undetected fungi, such as *Saccharomycetales* sp., *Debaryomyces hansenii* and some uncultured fungi.

**Conclusions**

To the best of our knowledge, this is the first study to analyze the diversity of YLS in BPH by using the PCR-DGGE system. This methodological approach can be used to assess the relationship between variation in planthopper performance and YLS community.

**FEMS-1675**

**Insect-microbes interactions**

## **GENETIC BASIS OF CYTOPLASMIC INCOMPATIBILITY CAUSED BY CARDINIUM**

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### **Background**

Endosymbionts of arthropods that cause cytoplasmic incompatibility (CI) manipulate host reproduction: bacteria in infected males sabotage the reproduction of uninfected mates, such that the relative fitness of uninfected females is depressed. *Cardinium* (phylum: *Bacteroidetes*) is the only bacterial lineage besides *Wolbachia* known to cause CI.

### **Objectives**

Identify genes involved with CI and host interactions by transcriptional profiling of a CI-inducing *Cardinium* in male and female parasitoid wasps (*Encarsia pergandiella*) and genome sequencing of four *Cardinium* strains causing different phenotypes.

### **Methods**

RNA was isolated from 1-3 day old male and female *Encarsia pergandiella* and bacterial mRNA was enriched using the Ribo-Zero™ Magnetic Gold Epidemiology Kit. Female and male samples were sequenced with Illumina Hi-Seq. The transcriptome was analyzed by mapping reads against the *Cardinium hertigii* genome. *Cardinium* genomes were also sequenced with Illumina HiSeq, assembled using reference-based and *de novo* assembly strategies and annotated using RAST.

### **Conclusions**

First genome comparisons revealed evidence for distinct differences between *Cardinium* strains causing different phenotypes such as the absence of the biotin synthesis pathway in parthenogenesis-inducing *Cardinium*. Currently, the metatranscriptome is analyzed in more detail, focusing on sex-specific gene expression of candidate proteins for CI and eukaryotic cell cycle regulation. A ubiquitin-specific protease and a ubiquitin ligase, genes of the putative anti-feeding prophage, and other genes involved in host cell interaction were highly expressed. We expect that the results of the transcriptome sequencing and comparison of *Cardinium* genomes causing different phenotypes will reveal first deep insights into the mechanisms of reproductive manipulation in *Cardinium*.

**FEMS-1504**

**Insect-microbes interactions**

## **DIETARY AND PHYLOGENETIC DETERMINANTS OF GUT COMMUNITY STRUCTURE IN HIGHER TERMITES**

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### **Background**

The microbial symbionts of termites play critical roles in the digestion of lignocellulose. Unlike wood-feeding lower termites, which are associated with cellulolytic flagellates, higher termites harbor an entirely prokaryotic gut microbiota. Simultaneously, they have considerably extended the range of their diet to lignocellulosic plant litter in various stages of humification. Since the same dietary specializations have evolved in different evolutionary lineages, higher termites offer the unique opportunity to study potential drivers of microbial community structure in the intestinal environment.

### **Objectives**

To assess the influence of host phylogeny and diet on the composition of the termite gut microbiota.

### **Methods**

We analyzed the bacterial microbiota in the hindgut of 19 higher termite species from different feeding guilds using Illumina sequencing of amplified 16S rRNA genes. Sequence reads were taxonomically classified using a curated reference database and subjected to phylogenetic and statistical analysis.

### **Conclusions**

The high similarity in the bacterial gut microbiota among the wood-feeding and humivorous members of different host lineages identified diet as a strong determinant of microbial community structure in higher termites. At higher taxonomic resolution, however, individual bacterial taxa showed a strong specificity for certain host groups, suggesting they are coevolving with their respective hosts. Nevertheless, evidence of co-cladogenesis is scarce and most bacterial lineages may not co-speciate with their respective hosts over a longer evolutionary time. Rather, the observed patterns of

host restriction seem to be enforced by a combined selection by microhabitat and ecological niche, enhanced by a vertical transmission of symbionts facilitated by the social lifestyle.

**FEMS-2295**

**Insect-microbes interactions**

**Δ-ENDOTOXINS OF BACILLUS THURINGIENSIS FORM AMYLOID FIBRILS  
THERE ARE INVOLVED IN THE FORMATION OF PARASPORAL CRYSTALS**

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<sup>2</sup>*Genetic, Research Institute for Genetics and Selection of Industrial Microorganisms, Moscow, Russia*

**Background**

The δ-endotoxins of the entomopathogenic bacterium *Bacillus thuringiensis* are multifunctional, so they are not only with highly specificity destroy invertebrate sensitive cells, but have an antimicrobial effect, leading to the destruction of cell walls and cytoplasmic membranes of some microorganisms like activities of others antimicrobial peptides and proteins (1, 2). We discovered for the first time the amyloid fibrils (AF) relating with parasporal crystals of *B. thuringiensis*.

**Objectives**

The crystals of *B. thuringiensis* subspecies: *kurstaki*, *israelensis*, *amagiensis* were the objects of our study.

**Methods**

Crystals and AF we studied using transmission electron microscopy. To confirm the formation of AF observed their interaction with the dye Congo red in the polarization interference microscope. Molecular mass of proteins was determined by PAGE electrophoresis.

**Conclusions**

δ-Endotoxins are capable to forming the AF having different thicknesses (like the previously studied AF). The degree of ordering of the AF increased near the crystals. Our research have given us reason to believe that the *B. thuringiensis* AF are involved in the formation of parasporal crystals. We discuss the ecological role and practical importance of these AF.

References. 1). Yudina T. et al., 2003. Can. J. Microbiol. 49(1). 37 - 44. 2). Yudina T. et al., 2007. Anaerobe. 13(1). 6 – 13.



**FEMS-1900**

**Insect-microbes interactions**

## **TARGETING GUT MICROBES: NOVEL APPROACH FOR INSECT CONTROL**

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### **Background**

Several insects are significantly affecting global economy through insect borne diseases in Agricultural and public health sector. Developing resistance towards frontline insecticides is the major challenge in insect control. Metabolic resistance is one of the major forms of resistance mechanisms. Role of insect gut microbes in supporting normal growth and development of insect is well documented. However, the role of insect gut microbes in developing resistance & favoring insect adaptation has not yet been deeply investigated. Targeting insect gut microbes could lead to unforeseen avenues for effective management of harmful insects.

### **Objectives**

To development novel strategy of insect control by targeting insect gut microbes.

### **Methods**

Two model insects are used in the present study malaria vector *Anopheles stephensi* and cotton pest mealybug *Maconellicoccus hirsutus*. Insect guts were cured with selected antibiotic treatment. These cured mosquito larvae's and Mealybug were observed for pesticide susceptibility. *M. hirsutus* were observed for fecundity, crawler and adult development wax content. While *An. stephensi* were tested for altered susceptibility against *Bacillus thuringiensis israelensis* treatment

### **Conclusions**

We observed that gut microbes cured mosquito larvae and Mealy bug became more susceptible to pesticide and environmental factors. Hence we put forward interesting effective vector or pest control strategy by targeting gut microbes.



**FEMS-2087**

**Insect-microbes interactions**

**MICROBIAL DIVERSITY IN GALL-INDUCED AND NON-INDUCED APICAL BRANCHES OF HAPLOPAPPUS FOLIOSUS (ASTERACEAE)**

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**Background**

Plant galls are abnormal vegetative growths that involve the participation of insect, bacteria, fungi and nematodes to induce growth. The role of microbial associations in gall formation are however, unknown.

**Objectives**

Our objective was to describe and compare the culturable diversity of bacteria and fungi from galled and non-galled apical branches in *Haplopappus foliosus*, an endemic flowering plant of the Asteraceae from Chile.

**Methods**

Fungal and bacterial isolates were grown in Sabouraud and enriched nutrient broth respectively. Molecular identification of the isolates was performed by analysis of 16SrRNA, 18SrRNA and ITS DNA genes analysis. Bioactive fungal secretions were analysed by HPLC-MS.

**Conclusions**

Our results indicate that there was a significant difference in microbial communities associated with cecidia and non-galled tissue, comprising 72% and 28% respectively. According to the Margalef index (10.25 galls and 1.95 non-galled) there was a high presence of fungi in galls 52% (n=31), bacteria 43% (n=26) and yeast 5% (n=3). Interestingly, some bacterial isolates have been described as PGPR, such as *Massilia sp.* and *Bacillus simplex*, and cellulose degrading *Cellulomonas denverensis*. Fungal isolates, have been characterized by their capacity to secrete bioactive compounds and we are currently using analytical techniques to determine potential function of these secretions in the formation of the gall association.

Knowing the microbial composition in galled and non-galled tissue will allow us to identify potential key roles in the formation of cecidia structures and develop future biotechnological strategies to prevent gall formation in endemic plants of the region.

## **FEMS-2540**

### **Insect-microbes interactions**

#### **MICRO-ASSOCIATIONS IN ANHYDROBITIC INSECT**

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<sup>4</sup>*Open Lab "Extreme biology", Kazan Federal (Volga Region) University, Kazan, Russia*

#### **Background**

There are organisms of different complexity, which can survive complete water loss conditions. Among them are bacterial and fungal spores, plant seeds, nematodes, rotifers, tardigrada. The most complex known organism with ability to withstand severe dehydration is larvae of African chironomid *P. vanderplanki*. During water depletion, larvae cells pump out water and accumulate trehalose and a number of protective proteins – Lea, Hsp, thioredoxins, etc.

#### **Objectives**

Here we attempted to investigate the bacterial community inhabiting the gut of sleeping chironomid *Polypedilum vanderplanki*, an insect able to survive complete dehydration by induction of special metabolic state anhydrobiosis.

#### **Methods**

To assess the variability of gut microbiota in *P. vanderplanki* larvae, we performed 454 pyrosequencing of 16S rRNA gene.

#### **Conclusions**

Comparison of larvae maintained under controlled laboratory conditions for more than 10 years and freshly collected from wild allowed to identify the core microbiota. Interestingly, representatives of larvae core microbiota were absent in soil samples suggesting true associations with host. By production of germ free larvae we showed that the absence of bacteria does not affect the rate of successful anhydrobiosis. However, sterile animals are suffering from the fungal infestation. We revealed that some isolated bacterial strains have antagonistic activity against fungi. Moreover, we found that during rehydration millions of virus-like particles are accumulating in the interspace between gut epithelium and peritrophic membrane. These viruses could promote the development of anhydrobiosis phenomenon in *P. vanderplanki* insect. The work is performed according to the Russian Government Program of Competitive Growth of Kazan Federal University.



**FEMS-1412**

**Insect-microbes interactions**

**METABOLIC ACTIVITIES OF STRICTLY AND FACULTATIVELY ANAEROBIC GUT BACTERIA AND THEIR INTERACTIONS IN A GERM-FREE COCKROACH MODEL**

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**Background**

The diverse microbial communities in the guts of termites and cockroaches represent a complex metabolic network of individual populations. In view of the special microenvironmental conditions within the gut and possible interactions among the microbiota, it is essential to investigate in how far the metabolic properties of pure cultures reflect their activities in their natural environment.

**Objectives**

To develop a protocol for the inoculation of germ-free cockroaches with defined cultures of autochthonous gut bacteria and to compare the metabolic profiles of the isolates in pure culture (*in vitro*) with their activities and interactions in the gut environment (*in situ*).

**Methods**

Pure cultures of gut bacteria were isolated from the cockroach *Shelfordella lateralis*. After inoculation of germ-free cockroaches, the strains were quantitated and localized *in situ* using qPCR, GFP-fluorescence and FISH. Gut conditions and metabolites were analyzed using microsensors, HPLC and GC.

**Conclusions**

The germ-free cockroach model provides first insights into the factors affecting the metabolism of the gut microbiota in their native environment. The strictly anaerobic *Fusobacterium* sp. (strain FuSL) and the facultatively anaerobic enterobacterium (strain EbSL) exclusively colonized the hindgut. Both strains showed high cell densities in mono-association, but the abundance of strain FuSL was much lower when co-inoculated with strain EbSL. Oxygen strongly influenced the metabolic products both under *in vitro* and *in situ* conditions. The availability of oxygen in the gut would also explain why the anaerobic strain FuSL is outcompeted by the

facultatively anaerobic strain EbSL, which should achieve higher growth yields under microoxic conditions at the gut wall.

## **FEMS-1215**

### **Insect-microbes interactions**

#### **EVALUATION OF ANTIBACTERIAL EFFECT OF AMERICAN COCKROACH HEMOLYMPH ON SOME NOSOCOMIAL PATHOGENIC BACTERIA**

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#### **Background**

Insects due to their long evolutionary history are able to recognize many pathogenic microorganisms and defend against them. Today, due to the development of resistance to synthetic antibiotics, researchers are trying to apply insect immune-derived products.

#### **Objectives**

This study was planned to investigate the antibacterial effect of the American cockroach (*Periplaneta Americana*) hemolymph on susceptible and resistant strains of nosocomial bacteria.

#### **Methods**

Adult cockroaches were reared in insectarium ( $25 \pm 2$  and 60% humidity) .To stimulate their immune system,  $20 \mu\text{l}$  of  $10^6$  *Escherichia coli* cells were injected into coelom .After 6 hours ,induced hemolymph were collected . The antibacterial effect of extracted hemolymph were assayed in the agar diffusion method on susceptible and resistant bacteria to antibiotics of ceftazidime, imipenem and, methicillin including, susceptible *E. coli* strain "ATCC 25922"& "PTCCIBRC-M 10708", *Staphylococcus aureus* "ATCC25923", *Pseudomonas aeruginosa* "ATCC 27853" and, resistant strains of *E.coli*, *Pseudomonas aeruginosa* and, *Staphylococcus aureus* that were isolated from the hospital environment and stored in bank of bacteria. Also, non-induced hemolymph effect was evaluated on the same bacteria.

#### **Conclusions**

Evaluation of induced hemolymph effect on types of strains showed that induced hemolymph affected about 75% of susceptible bacteria strains ( $P\text{-Value} < 0.001$ ), whereas it had no effect on resistant strains. Among bacterial tested, ceftazidime-sensitive *E. coli* ( PTCC ( ,ceftazidime- sensitive *E. coli* (ATCC 25922), and methicillin-sensitive *S. aureus* (ATCC 25923) showed sensitivity to induced hemolymph ( $P\text{-Value} < 0.001$ ).

These results showed that stimulation of immunity system American cockroach leading to production of antibacterial proteins and peptides which have inhibitory effect on bacterial depending on the bacterial strains and their sensitivity.



**FEMS-0866**

**Intracellular survival**

**THE ACTIVATION OF THE AUTOPHAGIC PROCESS BY EIEC PROVIDES A SMALLER SPREAD IN THE HOST CELLS.**

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**Background**

Autophagy has been described as an intrinsic host defense system for recognizing and eliminating intracellular-invading bacterial pathogens. However, invading pathogens have evolved mechanisms to avoid autophagic detection. Enteroinvasive *Escherichia coli* (EIEC), an important diarrheagenic *Escherichia coli*, are closely related to *Shigella*, showing remarkable phenotypic and genotypic similarities. However, the disease induced by EIEC is generally less severe than that induced by *Shigella* spp.

**Objectives**

EIEC express much less *icsB* than *S. flexneri*, our hypothesis is that EIEC are being efficiently recognized and eliminated by the host cell autophagic process. Moreover, we examined the role of *icsB* in this process.

**Methods**

We generate an *icsB* EIEC mutant by pGEM-T easy Vector System, pJP5603 plasmid and DH5- $\alpha$ pir system. The EIEC $\Delta$ *icsB* was confirmed by RT-PCR. HeLa cells were challenged with wild type EIEC and EIEC $\Delta$ *icsB* for different time points. The delipidation of LC3B was analyzed by Western Blot. Bafilomycin was used as a positive autophagy control.

**Conclusions**

Differently from *Shigella*, EIEC induce autophagy in HeLa cells and this process seems to be independently of *IcsB*. Our results suggest that other virulence factors than *IcsB* are involved in the autophagic process induced by EIEC and that *IcsB* seems to not be a mechanism of EIEC camouflage against autophagic recognition. Additionally, the activated autophagic process could be involved with the slightest spread of EIEC in the host cell, providing a better control of infection.



**FEMS-0789**

**Intracellular survival**

**SECRETORY PROSTATE APOPTOSIS RESPONSE-4 MIGHT REGULATE  
INTRACELLULAR SURVIVAL OF MYCOBACTERIA**

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**Background**

Regulation of apoptosis has become one of the hot topics in mycobacterial infection. Recently, prostate apoptosis response-4 (Par-4), a tumor suppressor protein, is known to cause apoptosis in prostate cancer cells. Par-4 also participates in apoptotic pathway via interaction with glucose regulated protein 78 (GRP 78), which is activated by endoplasmic reticulum (ER) stress under stressful situation. Previously, we reported that ER stress-induced apoptosis plays a critical role as a host defense mechanism against *Mycobacterium tuberculosis* (Mtb). In this study, we investigated the role of Par-4 during mycobacterial infection.

**Objectives**

The aim of this study was to investigate the effect of Par-4 expression on mycobacterial apoptosis in macrophages.

**Methods**

We showed that mycobacterial infection induced expression of Par-4 and GRP78. Additionally in this study, strong interaction between Par-4 and GRP78 was shown at Mtb-infected macrophage surface through quantitative colocalization analysis. To determine if apoptosis could be initiated by GRP78 and Par-4 complex, we used siRNA for Par-4 before Mtb infection. Annexin V staining was used to identify apoptotic cell death. As expected, siPar-4 decreased apoptotic cell death induced by mycobacterial infection.

**Conclusions**

We found that Mtb infection activated Par-4 expression as well as ER stress induction. The interaction of extracellular Par-4 and cell surface GRP78 led to apoptosis via activation of caspase-8/caspase-3 pathway. The intracellular survival of

Mtb H37Ra was increased in Par-4 siRNA treated macrophages. These data suggested that Par-4 plays a crucial role in the intracellular survival of mycobacteria.

**FEMS-0650**  
**(Human) microbiome**

## **INITIAL MICROBIAL COMMUNITY INFLUENCES GUT MICROBIOTA COMMUNITY STRUCTURE EVEN AFTER LONG-TERM DIETARY INTERVENTION**

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### **Background**

Short and long term dietary interventions influences the structure and activity of gut bacterial communities. Recent literature suggests that the impact of dietary changes depends on microbial community structure at the time dietary changes are initiated.

### **Objectives**

Investigate the effects of long-term dietary fibre intervention on the gut microbiota of rats from the same and different litters.

### **Methods**

Forty-five, 21-day old female rats from six different litters were fed one of three diets: 5% fibre, 26% fibre (insoluble-cellulose) and a diet consisting of 50% of a basal diet and 50% cooked red kidney beans (rich in soluble-fibre). Each animal in a litter was randomly assigned to one of the three diets. Gut microbiota composition was determined before and after 14-weeks of treatment. Microbial communities were characterized using 16s amplicon sequencing. Taxonomic affiliations were determined using Mothur and SILVA bacteria database.

At the start of the experiment the microbial communities present in a rat varied with a rat's litter membership. After 14 weeks on the experimental diets an animal's microbial community composition significantly varied with diet, but significant litter membership effects remained. After diets treatments fibre, short-chain fatty acid, nitrogen and carbon composition of the faeces depended on an animals litter membership and diet.

### **Conclusions**

The results suggest that efforts to enhance the health of humans and other animals through the use of prebiotics may have limited success in general due to among individual differences in the composition of their microbiotas and differences in how these microbiotas respond to dietary manipulation.



**FEMS-0632**  
**(Human) microbiome**

## **ENTEROCYTE-ASSOCIATED MICROBIOME OF THE HADZA HUNTER-GATHERERS**

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### **Background**

In a recently paper (Schnorr et al., 2014), we provided the first characterization of the fecal microbiota for a community of human hunter-gatherers, the Hadza of Tanzania. Our data suggested a new mutualistic layout for the gut microbiome (GM), with an enrichment in opportunistic bacteria and a depletion in health-promoting *Bifidobacterium*.

### **Objectives**

To obtain a deeper understanding of the GM-host mutualism in the Hadza, we explored the enterocyte-associated microbiome (EAM), whose layout of the EAM can inform about the functional influence on the host epithelium and other cell types in the mucosa, with critical implications for the maintenance of immune homeostasis. Microorganisms that directly interact with the enterocyte surface have a primary role in the microbiota-host cross-talk.

### **Methods**

By means of a previously developed non-invasive *ex-vivo* minimal model, based on mucus-secreting HT29 cells (Centanni et al., 2013; 2014), we characterized the EAM of 21 Hadza compared to 9 urban living Italians by 16S rRNA barcoded sequencing, and inferred the functional profiles via PICRUST.

### **Conclusions**

Compared to Italians, the Hadza EAM were characterized by a greater amount of adhesive and opportunistic microorganisms, such as Enterobacteriaceae and Pseudomonadaceae, resulting in a functional enrichment in cell motility, signal transduction, interaction and biofilm formation. Our results depict an interesting mutualistic configuration of the intestinal mucosal microbiome in Hadza that is

capable of enhanced microbiota-host cross-talk and interaction at the mucosal surface. These findings stress the importance of a deep microbe-host interaction at the intestinal mucosal interface along the course of human evolution.



**FEMS-1634**  
**(Human) microbiome**

**EFFECTS OF LINSEED AND HEMP SEEDS DIET SUPPLEMENTATION ON CAPRINE RUMEN BACTERIAL DIVERSITY.**

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**Background**

The rumen, an heterogeneous microbial community including bacteria, archaea, protozoa and fungi, is a natural habitat that has evolved into an efficient system for lignocellulose degradation. Thanks to this microbiota, ruminants convert the plant materials into digestible compounds, such as volatile fatty acids, their main energetic fuels, and bacterial proteins. The equilibrium of ruminal microbiota is dependent on the diet, which carries fermentation substrates, and the efficiency of ruminal microbiota can be strongly affected by dietary changes.

**Objectives**

The objective of this study was to assess the effects of linseed or hemp seeds diet supplementation on bacterial diversity in the rumen content of goats using the Next Generation Sequencing.

**Methods**

In nine pluriparous Alpine goats fed the same pre-treatment diet for 40 days, ruminal fluid samples were collected, before feeding, using an aesophageal polyethylene probe. After this pre-treatment period the goats were arranged to three dietary treatment groups consisting of control diet (C), control diet supplemented with linseed (L) or hemp (H) seeds. Ninety days later, the same ruminal sample collection procedure was performed. The bacterial DNA was extracted using a protocol described in literature and 16S rRNA gene amplicons on V3-V4 region analyzed by Miseq (Illumina).

## **Conclusions**

In the three dietary treatment groups, bacterial community was dominated by Bacteroidetes and Firmicutes with a high abundance of Prevotellaceae, Porphyromonadaceae and Veillonellaceae and a low presence of Ruminococcaceae and Lachnospiraceae. However, L treatment seemed to affect the bacterial population, reducing the microbial diversity.

**FEMS-3064**  
**(Human) microbiome**

**SEQUENCE BASED COMMUNITY ANALYSIS OF POLYMICROBIAL INFECTIONS**

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**Background**

Many microbial infections involve formation of biofilms containing multiple species. Besides the difficult treatment, the taxonomic and functional characterization of the microbial communities is a major challenge and classic culture-dependent methods often fail to fully characterize the infection.

**Objectives**

Our aim is the development of Next-Generation Sequencing based methods combining analysis of 16S-amplicons with metagenomics to reveal both the taxonomic and functional diversity of infection related microbial communities with a focus on virulence and presence of resistance determinants.

**Methods**

We analyzed samples from chronic lung infections and acute infections of dental implants (peri-implantitis). Taxonomical composition was determined by sequencing barcoded amplicons of the 16S rRNA V1-V2 variable regions using Illumina technology. Sequences were compared with the Ribosome Database Project for the characterization of taxa. Selected samples were also used to isolate whole genomic DNA for metagenomic sequencing using Illumina technology. Sequences were assembled and annotated by bioinformatic gene prediction and alignment to the NCBI non-redundant nucleotide database.

**Conclusions**

The taxonomic composition of the samples both from chronic lung infections and peri-implantitis revealed complex communities in both settings. The dominant taxa included pathogenic taxa like *Pseudomonas*, *Staphylococcus* and *Streptococcus* in the cystic fibrosis lung infections and several Bacteroidetes like *Tannerella* and *Porphyromonas*, which are commonly described in such infections. Analysis of Metagenomic sequences is ongoing to reveal the functional content of the communities with focus on virulence factors and resistance determinants. Employing metagenomics and amplicon sequencing combines taxonomic accuracy and cost-efficiency with a deep insight in the functional genomic content of microbial communities.

**FEMS-2036**  
**(Human) microbiome**

**ANALYSIS OF MICROBIAL DNA SIGNATURES IN THE RODENT NARES AND OLFACTORY BULBS.**

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**Background**

The nose is rich in bacterial species that produce outer membrane vesicles (OMV) known to participate in inter- and intra-species signalling. These structures, however, are highly reminiscent of eukaryotic exosomes and synthetic liposomes: all of these nanostructures involve lipid bilayers containing biomolecules such as DNA, RNA and/or protein. The blood-brain barrier has long scuppered efforts of drug delivery to the brain. Encouragingly, studies have revealed that the brain can be accessed across the olfactory nasal epithelium with biotherapeutics and nanoparticles.

**Objectives**

The main aim was to investigate the hypothesis that 1) the bacterial material is present in the brain of healthy rodents without causing disease and that 2) the possible entry route is via the nasal cavity across the olfactory epithelium. In addition, we analysed the microbial DNA signatures at the phylum and class level.

**Methods**

We first attempted PCR amplification of the V3 hypervariable region of bacterial rRNA genes from the mice and rats brain DNA. Produced amplicons were the subjects of 16S phylogenetic analysis by Ion Torrent Next-Generation Sequencing and RDP analysis.

**Conclusions**

The study proved existence of microbiomes in healthy mammalian brains. The comparison of the microbial signatures indicated that at least some of the bacterial DNA is common at both tissue loci, in support of the precept that nasal bacteria or their products might enter the brain through the olfactory epithelium. In addition, there is some similarity of the bacterial species between mice and rats, though some differences were down to host-species intricacies.



**FEMS-1687**  
**(Human) microbiome**

**METAGENOMIC CHANGES IN THE GUT OF PROTEIN ENERGY MALNUTRITION (PEM) MURINE MODEL**

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**Background**

Protein energy malnutrition is a potentially fatal body depletion disorder. The catastrophic effects of malnutrition include diarrhoea, malabsorption, increased intestinal permeability and alleviated immune response, thereby aggravating other pathological conditions and contribute to the global disease burden. Since effective treatment for malnutrition is lacking, therefore, metagenomic procedures allow to access the complex cross-talk between the gut and its microbial flora and understand how a different community composition affects various states of human health.

**Objectives**

The aim of the study is to develop protein energy malnourished (PEM) mice model and to evaluate the various changes in the body and organ weight, histological changes and the total protein content as compared to control. Moreover, a metagenomic approach has been employed for analysing the differences between gut microbial communities obtained from malnourished and apparently healthy mice.

**Methods**

Twelve mice were assigned to two groups (6 each/group) and fed either a malnourished (protein=2%, energy=295.2 calories) or a normal (protein=16%, energy=397.2 calories) diet. Body weight was taken till four weeks after which they were sacrificed. Weight of the visceral organs weight and their histological parameters were analysed. Faecal samples were further analysed for the differences in microbial communities between the two groups through RT-PCR. Serum samples stored under refrigerated conditions (-20°C) were also analysed for total protein content between both the groups.

**Conclusions**

The present study reveals the development of PEM model by analysing various morphological, anatomical and histological parameters. Moreover, it characterizes the microbial community resident in the gut of malnourished mice as compared to control.

**FEMS-3000**  
**(Human) microbiome**

**MUCOSA-ASSOCIATED MICROBIOTA IN THE SNOW TROUT INTESTINE (SCHIZOTHORAX ZARUDNYI, NIKOLSKII, 1897) ARE DIFFER FROM LUMEN-ASSOCIATED MICROBIOTA, AS REVEALED BY 454 PYROSEQUENCING**

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**Background**

As in mammals, studies on the intestinal microbiota of fish have revealed that the bacterial community may influence growth, feed conversion, epithelial development, and immunity of the host.

**Objectives**

This study aims at applying 454 pyrosequencing approach to study the autochthonous and allochthonous-associated microbiota from snow trout.

**Methods**

In total, 32 genomic DNA samples were obtained and then Roche 454 pyrosequencing was applied to the pooled set of 16S rRNA gene amplicons. Sequence data were prefiltered and analyzed with the Quantitative Insights Into Microbial Ecology (QIIME) v 1.8.0 pipeline using default parameters.

**Conclusions**

From our analyses, distinct microbial communities were detected between the snow trout gut-associated and intestinal content-associated microbiota, as has also been observed in human, pig, mice and alligators. According to the results Firmicutes phylum members composed the prominent phyla of the mucosa-associated microbiota regardless of the fish gender. However, in luminal-associated microbiota Fusobacteria phylum group made up the most dominant group in intestinal content of male and female fish. This suggests that the abundance and diversity of bacterial populations in gut mucus is, in general, quite different from the microbiota in gut contents, indicating that some microbial species poorly colonise gut mucosa layer. Many of these bacteria might be of high physiological relevance for snow trout as these groups have been implicated in vitamin production, nitrogen cycling and carbohydrate fermentation.



**FEMS-1589**  
**(Human) microbiome**

**DETECTION OF SAPOVIRUS GV.2 BY THE NEXT GENERATION SEQUENCER IN THE STOOL SPECIMENS OF PATIENTS OF GASTROENTERITIS OUTBREAK FROM WHICH PATHOGEN HAD NOT BEEN IDENTIFIED**

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**Background**

Causative pathogens have not been identified by ordinal examinations in some sporadic and outbreak cases of gastroenteritis received at Toyama Institute of Health. In contrast, some pathogens have been identified by metagenomic analysis using the next-generation sequencer (NGS).

**Objectives**

In this study, pathogens were searched by NGS in one outbreak of gastroenteritis from which pathogen has not been identified by conventional PCR analysis.

**Methods**

RNA-seq libraries were constructed from six specimens collected in the outbreak occurred in 3 facilities of health center for the elderly in April 2013, and were analyzed by MiSeq sequencer (Illumina).

**Conclusions**

About 20,000-50,000 reads were obtained in each specimen by NGS analysis, and 2 to 6 reads of nucleotide sequence of sapovirus (SaV) were identified from 3 specimens. These reads agreed with the part of the sequence of sapovirus Hu/Nagoya/NGY-1/2012/JPN (SaV GV.2) reported firstly in the food-borne gastroenteritis (Nagoya City in 2012) with 93-99% identity. Since multiple sequences of SaV were detected from the same specimen, the virus was suspected to be causative agent. Specific primers for SaV GV.2 were generated and PCR was performed. Products were obtained in all six samples by PCR, and their sequences

were revealed to be 99.8 % identity with that of SaV GV.2. Therefore, SaV GV.2 was concluded to be the causative agent of the present case.

Pathogens that are not able to be detected by conventional primers because of low homology may be identified by metagenomics analysis that is independent on specific primers as in this case.

**FEMS-0862**  
**(Human) microbiome**

**A 16S RRNA ANALYSIS OF SMALL INTESTINAL BACTERIA COMMUNITIES IN ADULTS WITH/WITHOUT ACTIVE CELIAC DISEASE**

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**Background**

Celiac disease (CD) is a chronic inflammatory disorder of the small intestine that presents only in a fraction of genetically predisposed individuals following gluten consumption. Recently, several studies have suggested that abnormalities in the small intestinal microbiota might be involved in the development or the pathogenesis of CD.

**Objectives**

The objective of this study was to characterize the specific composition of the duodenal microbiota of untreated CD patients and non-CD controls without mucosa damage in order to identify possible differences in bacterial communities in the upper small intestine.

**Methods**

Intestinal bacterial communities were identified by pyrosequencing of 16S ribosomal DNA extracted from duodenal biopsies. The study group included 9 untreated CD patients and 9 non-CD controls without duodenal mucosal atrophy.

**Conclusions**

Analysis of a total of 180,825 reads of the 16S rDNA showed that the majority of them were classified within two phyla: *Firmicutes* and *Proteobacteria*. Bacterial richness and diversity was higher in non-CD controls than in untreated CD patients. The principal coordinates analysis revealed that bacterial communities of non-CD controls and untreated CD patients were dispersed without forming a clear group according to the presence or absence of the CD. There is no characteristic pattern of bacterial communities associated to untreated CD patients. There are not differences in any bacterial group community frequencies in the upper small intestine between untreated CD patients and non-CD controls.

**FEMS-1665**  
**(Human) microbiome**

**METAGENOMIC ANALYSIS OF MICROBIOTA IN THE SEMEN OF PROSTATITIS PATIENTS: MOLECULAR APPROACHES TO ASSESS THE DIVERSITY OF MICROBIAL COMMUNITIES**

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Background

Prostatitis is one of the most common urological diseases in men and accompanied by bacterial infection in the prostate gland which occurs in 5-10% of prostatitis patients and also, frequently causes recurrent urinary tract infection. Urogenital health and diseases rely on the interaction between the host and the urogenital microbial community.

Pyrosequencing a next generation sequencing (NGS) technologies allow us to characterize microbial communities using bacterial 16S rRNA sequences with high magnitude faster than previous Sanger dideoxy chain-termination method. Therefore, the aim of this study was to identify specific microbial descents that may play crucial roles in the development of prostatitis and to examine whether the presence or abundance of these bacterial changes in semen of prostatitis patients.

Material and Methods

Semen samples were collected from prostatitis patients and extracted genomic DNA for pyrosequencing by using DNA extraction kit.

Results and Discussion

In this study, some abundant pathogenic genus, such as *Anoxybacillus*, *Staphylococcus*, *Corynebacterium*, *Pseudogluconobacter* and *Enterococcus* in the prostatitis sample were found by using the pyrosequencing techniques.

In this study, in case of normal control, *Streptococcus* and *Corynebacterium* were abundance to 33.5% and 21.2%. On the other hand, the most abundant genera in semen of prostatitis patient were *Enterococcus* (20.7%), *Pseudogluconobacter* (16.2%) and *Anoxybacillus* (14.3%). These data provided the comprehensive report on bacterial communities between normal and prostatitis patients and also, it is concluded that prostatitis is related to substantial alteration in the composition and representation of the urogenital bacterial biota.

This identified variability in community membership and structure has important implications for understanding the etiology of prostatitis, for developing diagnostic tools and for treatment.

**FEMS-1698**  
**(Human) microbiome**

**UNDERSTANDING OF HUMAN DANDRUFF SCALP THROUGH COMPARATIVE ANALYSIS BETWEEN MICROBIOME AND MYCOBIOME**

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**Background**

Dandruff is a global common problem, up to 50% of both genders suffer from dandruff. However, although bacteria and fungi associated with dandruff are known, understanding the etiology of dandruff is incomplete.

**Objectives**

Correlation of the bacterial and fungal communities with dandruff is needed because the human skin is associated with many microorganisms with potential for both beneficial and detrimental interactions.

**Methods**

For all cases, samples from normal scalps and severe dandruff patients were collected and analyzed under protocols approved by Chung-Ang University College of Medicine IRB (Protocol #2012-02-01). From the collected samples, we amplified variable regions V1-V3 of the 16S rRNA gene (bacteria) and the D1/D2 region of the 26S rRNA gene (fungi) using universal primers and GS-FLX pyrosequencing.

**Conclusions**

Firmicutes were the most dominant in dandruff patients and showed an increase from 5.2% in cases defined as normal scalps to 91.0% in severe dandruff patients. Whereas, Proteobacteria decreased from 60.1% in the normal scalp to 6.2% in dandruff patients. Similarly fungi of the phylum Basidiomycota were associated with the normal scalp, decreasing from 66.6% to 24.7% in severe dandruff. In the Firmicutes *Staphylococcus* spp. were dominant in the dandruff scalp. In contrast, *Malassezia* spp. in the Basidiomycota were the most abundant fungus in the healthy scalp. This switch from *Malassezia* to *Staphylococcus* provides new comparative information on the microbiomes of the normal scalp and patients with exacerbated dandruff. Consequently, our results can be expected to lead to improved diagnosis and treatment.

**FEMS-0540**  
**(Human) microbiome**

**GENOMIC ANALYSIS OF NOVEL CHITINOLYTIC SUBSPECIES OF  
CLOSTRIDIUM SARTAGOFORME FROM BUFFALO RUMEN REVEALS ITS  
IMPORTANT ROLE IN VOLATILE FATTY ACID PRODUCTION**

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**Background**

The rumen of herbivores is an open fermentation chamber for digestion of complex plantbiomass by highly efficient bacteria

**Objectives**

Isolation of anaerobic rumen microbes from buffalo and its pheno-genotypic analysis. Correlate the genomic analysis with metagenome data for implication of the isolate's role in rumen

**Methods**

A strain of *Clostridium sartagoforme* species was isolated from buffalo rumen, involved in cellulose and chitin degradation. The isolate was cultured between 37°C and 42°C temperature with an optimum pH of 7.5 in Hungate's medium supplemented with cellulose. Whole genome sequencing was performed using ion torrent PGM. Taxonomy study using local BLAST, showed highest phylogenetic relation with *Clostridium sartagoforme* DSM 1292 strain based on comparison of the 16S rDNA sequences. Comparison of sequences coding for housekeeping genes phosphoglycerate kinase and gyrase B confirmed its taxonomic novelty within the *Clostridium* genus. Biochemical assessment of strain's fermentation ability using AN-Biolog plate revealedthat the strain utilized more than 30 different metabolite precursors. Pathway analysis make known the organism to be highly active in the production of volatile fatty acids (VFAs).

**Conclusions**

The isolate represents a new subspecies, for which the name is proposed *Clostridium sartagoforme* AAU1 (GenBank Accession:ASRV00000000). Genomic analysis gives an insight about the role of bacteria in determining ruminant host physiology.VFAs produced by the isolate play a prominent role in animal health maintenance by contributing a major proportion of the ruminants' daily energy requirement. A deeper understanding of genes involved and enzymes encoded allow further industrial applications and modulation of the rumen for enhanced farming applications.

**FEMS-2593**  
**(Human) microbiome**

**METAGENOMIC ANALYSES REVEAL A CELLULOLYTIC AND FERMENTATIVE LIFESTYLE IN THE GUTS OF EPULOPISCIMUM-REPLETE SURGEONFISHES**

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**Background**

*Epulopiscium fishelsoni*, is a giant bacterium found exclusively in the guts of surgeonfishes. Three years after the publication of an incomplete draft genome of *Epulopiscium* sp. type B from *Naso tonganus*, we still have no clue into the metabolic potential of this hitherto uncultivated symbiont, and more so, the functions of the gut microbiota in reef fishes.

**Objectives**

Hence we were prompted to fill this knowledge gap, and thus provide a foundation for understanding the physiology and ecological roles of their microbiota.

**Methods**

We therefore employed a metagenome-sequencing approach to explore the phylogenetic diversity of *Epulopiscium*, and through binning of assembled metagenomic contigs, also shed insights into their metabolism.

**Conclusions**

Our data shows that distinctive feeding guilds of surgeonfishes harbour different *Epulopiscium*-like phylotypes, and that the low-to-high intestinal abundances of these bacteria correlate with their algal diet types. Metabolic reconstruction of the draft *Epulopiscium*-like genomes from these fishes revealed a capacity to catalyze hemicellulolysis and an anaerobic fermentation lifestyle that produces acetate and lactate. Our data thus provides the first in-depth analysis into their diversity and putative symbiotic role, which in turn illuminates the potential metabolic links shaping the symbiosis between the host and the giant bacteria. We further present more insights from the draft genomes, focusing on the nature of this symbiosis, and its interconnection to the trophic ecology of the fishes.



FEMS-2296  
(Human) microbiome

## ASSESSMENT OF THE EFFECT OF ROUGHAGE DIET ON FUNCTIONAL CAPACITY RELATED WITH THE METABOLISM OF AROMATIC COMPOUND OF BUBALUS BUBALIS (MEHSANI BUFFALO) RUMEN METAGENOME

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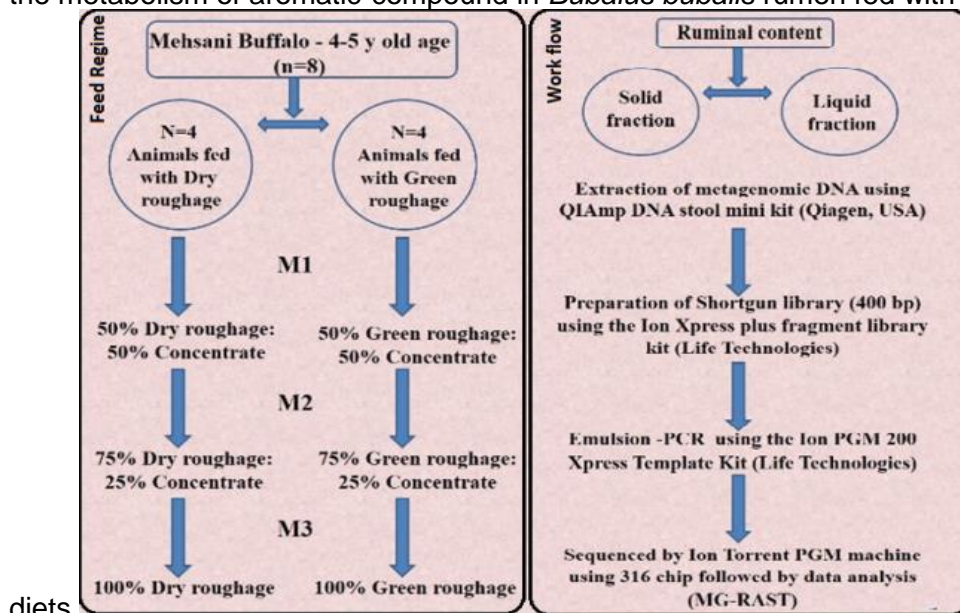
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### Background

Aromatic compounds comprise one-quarter of the Earth's biomass and are the second most widely distributed class of organic-compounds in nature, next to carbohydrates. In tropical-countries, the buffalo are fed on lignocellulosic agricultural by-products like cereal-straws and tree-foliage. Ruminants digest such plant-materials by microbial-processes. The role of microbiota as reservoir of its unique functional capacity related to the metabolism of aromatic-compound needs to be explored in buffalo rumen.

### Objectives

In the view of above concern, objective of the present study was to account the comparatives profiling of phylogenetic and functional-potential of genes related with the metabolism of aromatic-compound in *Bubalus bubalis* rumen fed with different



diets.

### Methods

The metagenomic analysis of liquid and solid-fraction of the rumen-biomaterial collected from *Bubalus bubalis* was carried out using high-throughput sequencing. The experimental animals received M1 diet for 6 weeks followed by M2 for 6 weeks and

then M3 for subsequent 6weeks. On the last-day of each experimental feeding period, rumen-samples were collected 3h post feeding using stomach tube.

## **Conclusions**

The work presented here describes composition of overall functional-capacity related with the genes for metabolism of aromatic-compound and responsible taxonomic-communities of buffalo rumen-ecosystem. The higher %abundance was found to be related with genes involve in peripheral-pathway for catabolism of aromatic-compound in all treatment-groups. The taxonomic analysis of metagenomic reads indicated that rumen-microbiomes were dominated by Bacteroidetes and Firmicutes phyla in the all treatments-groups. The information obtained from this research will open new-horizons towards a full understanding of functional-genes related with metabolism of aromatic-compound and metabolic-capabilities of complex-compound degrading rumen-microorganisms.

**FEMS-2067**  
**(Human) microbiome**

**INTAKE OF A LICHEN-BASED DIET ALTERS THE POPULATION OF  
METHANOGENIC ARCHAEA IN THE RUMEN AND HINDGUT OF REINDEER**

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**Background**

Reindeer are large Holarctic herbivores, which rely on a consortium of anaerobic microorganisms in the rumen and hindgut to digest plant cell wall carbohydrates. Reindeer may include a large fraction of lichens in their winter diet. Lichens are high in phenolic secondary compounds, which may affect the reindeer gut microbiome, and possibly alter the output of methane. Methane is produced by methanogenic Archaea mainly from CO<sub>2</sub> and hydrogen.

**Objectives**

In this project we examined the effect of lichens on the reindeer rumen and hindgut methanogenic Archaea.

**Methods**

Rumen and cecum samples were collected from two groups of Norwegian reindeer (*Rangifer tarandus tarandus*), one group (n=4) fed lichens and one fed a standard pelleted reindeer feed (n=3). The diversity of methanogens was assessed using 454 pyrosequencing. Real-time PCR experiments were performed to estimate the population densities. All animals were sacrificed in accordance with Norwegian regulations, Sections 9 and 10 in the Norwegian Animal Welfare Act.

**Conclusions**

Densities of methanogens and protozoa were higher in the cecum of lichen-fed reindeers but no differences were found in rumen samples. *Methanobrevibacter* (≥ 97% similarity) was the major genus of Archaea in all the samples, with *M. thaueri* as the dominant species in all reindeers fed lichens (>60% on average). Remarkably, *M. ruminantium* suffered a significant increase (up to 23,8%) with a lichen-based diet in both sampling sites. In conclusion, the intake of lichens affected to some extent the microbial diversity and density of the microbiome housed in the rumen and cecum of reindeer.



**FEMS-1448**

**Metagenomics in the environment**

**DRINKING WATER MICROBIOME: TOWARDS A METHOD ASSESSING  
MICROBIAL STRUCTURE VARIABILITY**

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**Background**

Drinking water quality is a public health concern worldwide. Growing evidences depict drinking water as a complex matrix, in which a wide diversity of microorganisms interact in a dynamic network. Recent studies reveal that drinking water treatment process can affect the microbiome structure. Moreover the occurrence of antibiotic resistance genes in water is becoming an issue of great interest as the mobile resistome can easily spread among species. Molecular techniques can give a deeper knowledge, going beyond the limit of culture-dependent methods.

**Objectives**

In this study we evaluated and standardized a new pipeline for microorganisms concentration, DNA extraction and amplification, suitable for molecular analysis and optimized for High-Throughput Sequencing (HTS) approaches.

**Methods**

We collected samples in a water treatment plant in Milan (Italy), at different steps of the potabilization processes, from the aquifer to the tap. We analyzed the presence and the relative abundance of bacteria and eukaryotic microorganisms across the water treatment plant. Furthermore the presence of specific antibiotic resistance genes was detected and quantified with Real Time PCR, at each step of water treatment process. Since molecular techniques are unable to differentiate between viable and nonviable microorganisms, live/dead ratio was estimated using SYTO9/propidium iodide staining coupled with microscopy visualization. These analyses are integrated in a broader study characterizing microbiome structure variability using HTS techniques, in order to better understand this complex ecosystem.

**Conclusions**

The results agree with those obtained in the few recent studies published till now and can help to unravel the dynamics underlying water microbiome changes.



**FEMS-0974**

**Metagenomics in the environment**

**BACTERIAL AND CYANOBACTERIAL METAGENOMICS IN THE EXTREME ENVIRONMENT FORMED BY GYPSUM AND HALITE INTERFACE IN A SALINE SOIL CRUST**

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**Background**

Phototrophic and heterotrophic bacteria have been found directly inside halite evaporites that formed as bottom-growth crusts in a hyperarid core and inside gypsum minerals. The role of microorganisms in the precipitation of authigenic minerals like carbonates, sulphides and silicates, has been documented by some authors in several natural systems.

**Objectives**

In this study the interface between biological and geochemical components in the surface crust of a saline soil was investigated. The bacterial community structure and diversity as well as the possible role of the photosynthetic microbial community in the formation of the soil crust was evaluated.

**Methods**

A variable pressure scanning electron microscope equipped with energy dispersive X-Ray diffraction analysis was used for geochemical analysis, while pyrosequencing of the V2-V3 16S rRNA gene region was used for characterizing microbial diversity and community structure.

**Conclusions**

The organization and diversity of the microbial taxa was analyzed according to the physical and chemical heterogeneity of that peculiar habitat. Mineral structures made of gypsum and halite were documented as crystallized around the filaments of cyanobacteria. A univocal causal relationship could not be established between the crystallization of gypsum or halite and cyanobacterial or algal metabolism but their compartmentalization respect to the biological mat suggested that the organisms

could have at least a physical control over the distinctive structures produced. The results provided information on the type of distribution of different bacterial and cyanobacterial groups as a function of the biogeochemical interfaces, suggesting also possible forms of microbial interaction.



**FEMS-1355**

**Metagenomics in the environment**

**METAGENOMIC ANALYSIS OF MICROBIAL AND VIRAL COMMUNITIES WITHIN A FRESHWATER LAKE IN NORTHERN IRELAND**

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**Background**

Bacteriophages are the most abundant biological entities in the biosphere. They influence the evolution and dynamics of microbial populations. Although phage populations were studied extensively for the marine environment only few studies were dedicated to freshwater ecosystems.

**Objectives**

The main objective of this project is to study the phage population in Lough Neagh, the largest freshwater lake in the British Isles and to analyse interaction of these phages with bacterial hosts.

**Methods**

Both total microbial and viral subpopulations were investigated using standard protocols. Isolation of viral fractions was performed by common procedures. Pyrosequencing was used to identify bacterial communities and the dynamics of the bacterial population's changes in Lough Neagh are compared with seasonal variation. Illumina sequencing was used to characterise viral communities in the same environment.

**Conclusions**

16S rRNA analysis detected a diverse range of bacterial phylum in both water and sediment. Proteobacteria, Acidobacteria, Bacteroidetes and Planctomycetes are found in the freshwater samples which are comparable to the sediment samples. The water samples taken from Lough Neagh have bacteria phylum which are not present in the sediment samples such as Chlamydiae. Analysis of viral metagenomes detected the presence of 16S rRNA sequences probably originated from transducing particles. For example Pelagibacteraceae was identified and. this bacterial family is more commonly found in the oceans rather than a freshwater lake. Possibly this may indicate interactions between bacterial species from two different environments. Illumina analysis of viral metagenomes provided a diverse range of information and can be compared to other published metagenomes.

## **FEMS-2455**

### **Metagenomics in the environment**

#### **OPTIMIZED AND STANDARDIZED SINGLE INDEXING SOLUTION FOR HIGHLY MULTIPLEXED MICROBIAL COMMUNITY PROFILING ON ILLUMINA MISEQ PLATFORM**

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#### **Background**

The growing need to survey the tremendous microbial diversity in a culture independent manner, has led to the development of molecular methods through sequence profiling of conserved genes, in scientific field like ecology, agronomy, biotechnology, plant, animals or human health. Recently, the improvement of the Illumina MiSeq platform to a 2x300 bases paired-end version made it much more attractive for amplicons sequencing in metagenomics. Nevertheless, the lack of nucleotide diversity at initial sequencing positions due to sequence conservation is problematic on Illumina platforms. Moreover, validated Illumina amplicon sequencing protocols do not support more than 96 samples per run, which underutilized the overall capacity of a sequencing run.

#### **Objectives**

We therefore worked on the development of a standardized and optimized high-multiplexed metagenomic solution for the exploration of complex environments.

#### **Methods**

We developed an integrated solution combining (i) a custom and standardized amplification protocol for library preparation; (ii) a 192 custom single index validation strategy within a single run; (iii) the inclusion of a nucleotide sequence variability at the first sequencing cycles; (iv) an accurate paired-end reads assembly process and (v) a dedicated pipeline for taxonomic affiliation and microbial communities analysis.

#### **Conclusions**

Our high-multiplexing sequencing solution validated within complex metagenomics samples, provided high sequencing accuracy and technical reproducibility with no index biases based on taxonomic distribution analysis. The association of technical and bioinformatical improvements yields substantial cost reductions and provides greater target flexibility to investigate structure as well as functions of microbial communities through 16S rDNA, 18S rDNA, ITS and functional genes profiling.



**FEMS-2643**

**Metagenomics in the environment**

## **METAGENOMIC ANALYSIS OF NATURAL MICROBIAL COMMUNITIES IN ELECTRO-ACTIVE MARINE SEDIMENTS**

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### **Background**

In marine sediments, a novel group of filamentous sulfide-oxidizing bacteria has recently been discovered, which induce electrical currents over centimetre-scale distances. These so-called cable bacteria grow from the oxic zone into the sulfidic part of the sediment up to a depth of several centimetres. Cable bacteria use a radically novel microbial metabolism, in which electron donor and electron acceptor reactions are spatially separated: the oxidation of sulfide in anoxic sediment layers is coupled to the reduction of oxygen at the sediment surface. This mechanism is only possible when electrons liberated from sulfide oxidation are transported from cell to cell along the length of the filament to the oxic sediment surface.

### **Objectives**

To identify a marine sediment where cable bacteria are active in situ and to study the sediment microbial community using shotgun metagenomic sequencing.

### **Methods**

Depth profiles of oxygen, sulfide and pH were taken with microsensors and cable bacteria distribution with depth was evaluated using fluorescent in situ hybridisation. Metagenomic analysis was performed for different depths of the sediment: the oxic surface sediment, the suboxic zone where neither oxygen nor sulfide was present, and the deeper sulfidic zone without cable bacteria. Extracted sediment DNA was sequenced using Illumina MiSeq with 300 bp paired end reads.

### **Conclusions**

At the examined North Sea site, microsensor profiles indicated that metabolic activity of cable bacteria dominated the sediment geochemistry. The obtained metagenomic

data provide insight into the microbial community composition and functioning, and possible interactions between cable bacteria and other taxonomic groups.

**FEMS-1105**

**Metagenomics in the environment**

## **INFLUENCE OF INOCULA SLUDGE VOLUME INDEX ON ANAEROBIC GRANULATION**

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### **Background**

Granulation is important for successful start-up of high-rate anaerobic reactors and usually takes long time. The influence of inoculum's Sludge Volume Index (SVI) on granulation in high-rate upflow Hybrid Anaerobic Reactor (HAR) was studied.

### **Objectives**

Understanding of microbial community changes due to variations in SVI will help in providing novel reactor setting up conditions to obtain faster granulation rate in high rate anaerobic reactor.

### **Methods**

Ten HARs were operated with inoculum having different values of SVI (100 to 325), SVI were varied by adding different amount of inoculum (10% to 50% of the reactor volume). The methanogenic and bacterial community structures of anaerobic sludge obtained from these ten HARs were examined using culture-independent techniques like DGGE and qPCR.

### **Conclusions**

Results showed that granulation was achieved in HARs with SVI values ranging from 150-210 or 18% to 27% inoculum of the reactor volume. DGGE identified 17 methanogenic and 22 bacterial operational taxonomic units in the ten reactors' samples. DGGE analyses showed dominance of *Methanosaeta* and *Methanosarcina*-like species in reactors where granules were formed. qPCR analysis also confirmed that granulating reactors were rich in aceticlastic methanogens especially *Methanosaetaceae*. Bacterial rRNA genes obtained from DGGE results, were affiliated to four Orders -*Clostridiales*, *Lactobacillales*, *Actinomycetales* and *Bacteroidales*. But, no significant correlation was observed between granulation and bacterial abundance. The concentration of bacterial 16s rRNA genes was found to be less in granulating than non-granulating systems unlike methanogenic genes. Thus, this study suggests to make conditions favorable for growth of aceticlastic methanogens to decrease granulation time.

**FEMS-1429**

**Metagenomics in the environment**

## **MINING SLAUGHTERHOUSE DRAIN BIOFILMS FOR NOVEL ENZYMES AND BIOSURFACTANTS**

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### **Background**

Environmental DNA derived from uncultured bacteria has huge potential as a source of novel bioactive molecules as shown by recent screening campaigns of metagenome libraries which revealed a number of novel protein families, extremozymes and secondary metabolites. Before constructing a metagenomic library, the choice of the habitat should be carefully considered because it predefines the types and properties of biocatalysts to be discovered.

### **Objectives**

In this study, we have constructed a metagenomic library in *Escherichia coli* DH10b with DNA isolated from the microbial population of a slaughterhouse drain biofilm. By comparative screening for lipolytic, proteolytic and detergent activities we have identified number of clones producing bioactive molecules.

### **Methods**

By applying a functional screening assay for biosurfactant activity, two haemolytic and surface-active clones (SA343, SA354) were identified. Sequence and structural homology analyses revealed that they contained genes encoding N-acyl amino acid synthases (NAS) located on environmental DNA fragments.

### **Conclusions**

The respective NAS proteins (Nas343 and Nas354) were shown to be responsible for the haemolytic phenotype and the biosurfactant synthesis *in vivo*. The molecular organisation of the NAS domain linked here to a c-di-GMP binding domain has so far not been identified in any other known protein. The produced biosurfactant was purified and identified by NMR spectroscopy as N-myristoyl-tyrosine. Its biophysical properties including the critical micelle concentration and the ability to reduce surface tension were compared with chemically synthesised N-myristoyl-tyrosine and shown to be in a similar range. Interestingly, N-acyltyrosine also showed antibiotic activity against various Gram-negative and Gram-positive bacteria.





**FEMS-1232**

**Metagenomics in the environment**

**MICROORGANISMS INVOLVED IN CARBON AND NITROGEN CYCLING IN THE ARABIAN SEA OXYGEN MINIMUM ZONE**

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**Background**

Oxygen minimum zones (OMZs) play a major role in marine biogeochemical cycling. They are characterized by a high microbial diversity adapted to life along an oxic-anoxic gradient. In particular anammox and/or denitrification are dominant processes in these zones accounting for significant loss of nitrogen from the ocean. However, other processes within the nitrogen and also carbon cycling have not been explored well.

**Objectives**

To get insights into the organisms involved in carbon and nitrogen cycling in the Arabian Sea OMZ, we studied the meta-genomes of filtered ocean water recovered at two different depths, the upper zone (170m deep, PA2) and the central zone (600m, PA5).

**Methods**

We systematically screened for marker genes indicative for key nitrogen and carbon cycling processes by blasting the meta-genomes data against manually curated functional gene databases.

**Conclusions**

Both zones are characterized by a large fraction of alpha, gamma and delta-proteobacteria. In PA5, anammox contributed a significant amount to the overall diversity (about 8%) whereas Thaumarchaea were present in higher numbers in the upper zone (about 9%). The predominance of nitrifying archaea over bacteria could also be confirmed by *amoA* diversity. No bacterial *amoA* was detected in the entire dataset, and only very few sequences related nitrogen fixation were recovered. Furthermore, no canonical methane oxidizers could be found. However, *pmoA* sequences only distantly related to known diversity are present and could be linked to a published single cell genome, indicating the possibility of a cryptic methane cycle in the Arabian sea OMZ.

## **FEMS-2640**

### **Metagenomics in the environment**

#### **MICROBIOMES OF THE BUILT ENVIRONMENT ARE ALTERED BY DIFFERENT ROOM MAINTENANCE**

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#### **Background**

The excessive removal of microbes from controlled built environments like intensive care units, operation theatres and especially cleanrooms is daily routine. However effects of these stringent maintenance procedures on our surrounding microbiomes in indoor environments are unknown.

#### **Objectives**

Since recent studies showed the enormous potential of the human microbiome towards our health, this study aims to resolve the interactive interference of all microbiomes in the built environment and determines its potential for human well-being indoors<sup>1</sup>.

#### **Methods**

High throughput deep sequencing technologies were applied for shotgun metagenomes and 16S/18S rRNA and ITS gene amplicons of Archaea, Bacteria, Eukaryota and fungi. Viability assays using ATP (adenosine tri-phosphate) and PMA (propidium monoazide) were deployed to identify potential viable microbiomes and supported by qPCR to investigate microbial abundance<sup>2,3</sup>. The biotechnological potential was further characterized by VOC's (volatile organic compounds) assays of culturable indoor bacteria<sup>4</sup>.

#### **Conclusions**

These assays revealed high reduction of viable microbial abundance in cleanrooms<sup>5</sup>, higher similarities of viable microbiomes between controlled and uncontrolled areas compared to the total microbial fraction, distinct profiles of microbial communities from floors, medical devices and workplaces in an intensive care unit, and for all controlled built environments not only an overlap with the human, but also the plant microbiome. Hence plant leaves could be identified as a main source for microbial distribution on the surrounding indoor environment.

The knowledge of certain key species and their ecological key functions in controlled as well as uncontrolled built environments will help to control indoor environments in a more sophisticated way for our health inside buildings in the future.

**FEMS-0520**

**Metagenomics in the environment**

**ACIDOPHILIC AUTOTROPHS AND HETEROTROPHS FACILITATES SLUDGE STABILISATION WITH SIMULTANEOUS HEAVY METAL LEACHING**

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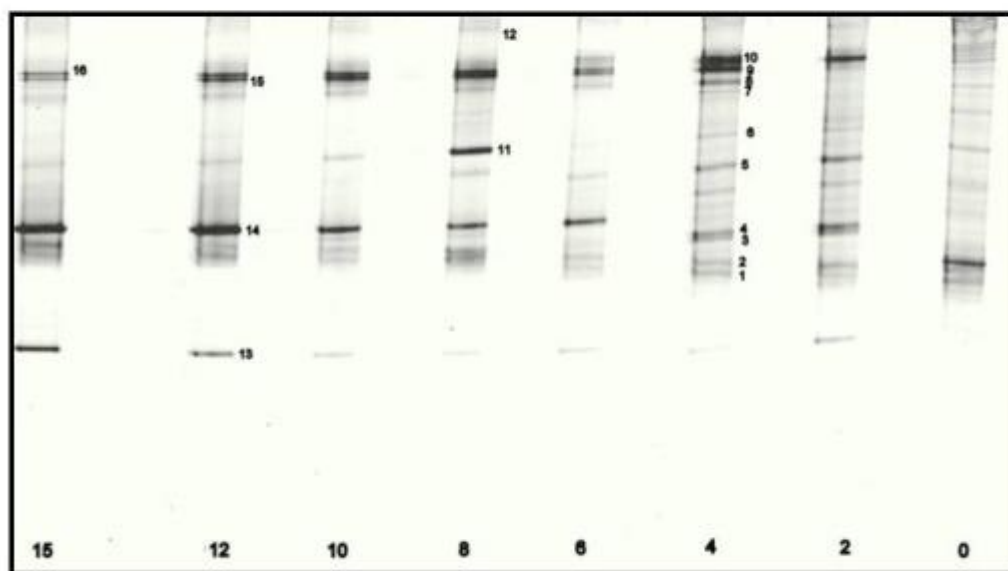
**Background**

Presence of heavy metals impedes use of sewage sludge as a fertilizer. Simultaneous sludge digestion and metal leaching (SSDML) combines conventionally distinct stages of metal leaching and sludge stabilisation into one, making the process economically attractive. Insights into the microbial community involved could enable further optimization of the process.

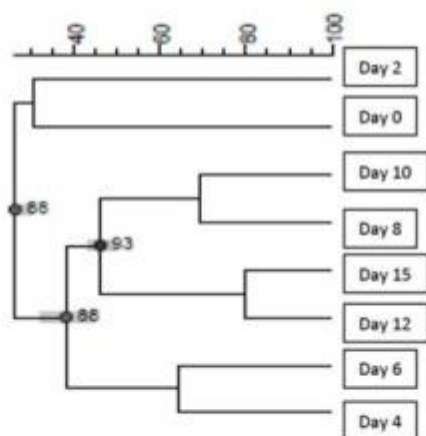
**Objectives**

To evaluate the performance of SSDML and understand corresponding microbial (bacterial) community shifts. .

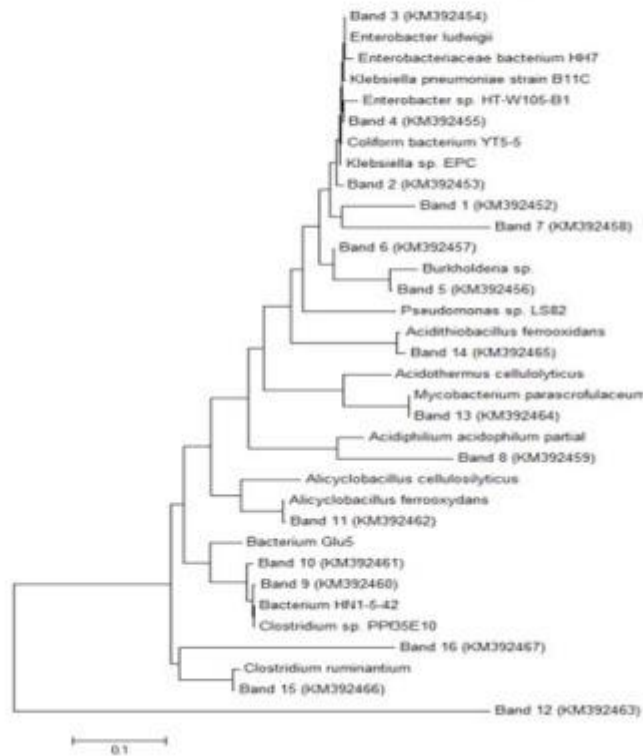
**Figure 1. Bacterial DGGE profile during the process (0, 2, 4, 6, 8, 10, 12 and 15 are the n<sup>th</sup> day of operation of experiment)**



**Figure 2. Unweighted pair group method with arithmetic mean (UPGMA) analysis of DGGE profile in Figure 1**



**Figure 3. Phylogenic relationship of bacterial 16S rRNA sequences obtained from bands eluted from gel shown in Figure 1**



## Methods

Batch study was carried out at 37°C for 15 days after adding sulfur to heavy metal contaminated sludge, with aeration rate of 0.6 vvm. Samples for heavy metals, volatile suspended solids (VSS) analyses and DNA extraction were collected regularly.

## Conclusions

Bio-oxidation of sulfur to  $H_2SO_4$  lowered the pH aiding leaching of metals. Zinc, chromium, cadmium and lead reduced by 86.75%, 64.26%, 79.17% and 69.58% in the dry sludge respectively. Concomitantly, VSS reduction of 49.40 % was obtained making the sludge stable.

*Acidithiobacillus ferrooxidans* and *Acidiphilium acidophilum* were found to be the main bioleaching microorganisms through 16S rDNA PCR-Denaturing Gradient Gel Electrophoresis (DGGE) analysis, in contrast to the commonly reported sulfur-oxidising *Acidithiobacillus thiooxidans*. *Enterobacter*, *Alicyclobacillus cellulolyticus* and *Pseudomonas* were the dominant heterotrophic organisms present across all samples. UPGMA analysis showed a significant microbial shift during the course of operation which could be mainly attributed to the change in pH and solids content.

Thus, after the single stage treatment of SSDML, sludge obtained was stabilised with reduced heavy metal load. PCR-DGGE analysis showed that both heterotrophs and bioleaching microorganisms were present and adapting well according to the dynamic pH.

**FEMS-1771**

**Metagenomics in the environment**

**QUALITATIVE AND QUANTITATIVE BIAS ESTIMATION OF MICROBIAL COMMUNITY ANALYSIS USING BARCODED 454 FLEX+ METHODOLOGY FOR CLAY CONTAINING GEOLOGICAL SAMPLES**

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**Background**

High throughput sequencing methodology can be used to determine the composition of environmental microbial communities. Generally, a barcoded PCR amplicon of the omnipresent 16S rDNA gene is used to unravel complex microbial communities. However, several biases and technical difficulties linked to this methodology, may jeopardize the outcome of the community analysis. For instance, it is often difficult to extract DNA out of environmental samples due to interferences caused by the geological matrix. This is especially true for clay containing geological samples. Beside, often containing highly reactive dissolved organic materials, clayey soils are also known for their high sorption capacity of (biological) macromolecules. Therefore, the presence of clay minerals may be responsible for additional biases when analyzing microbial communities of soil or geological samples.

**Objectives**

To quantify the biases, qualitatively and quantitatively, caused by the presence of clay minerals, during analysis of the composition of microbial communities in geological samples, with high throughput barcoded 454 Flex+ DNA sequencing methodology.

**Methods**

Four identical bacterial mockup samples were made, by mixing 18 different soil bacteria. Boom clay was only added to two samples. DNA was extracted, with an identical DNA extraction procedure. The 16S rDNA gene was PCR amplified with four different barcoded 16S<sub>341</sub>-forward primers and one 16S<sub>1492</sub>-reverse primer. The amplicons were sequenced using the 454 Flex+ DNA sequencing methodology. The data were treated with our in house developed bioinformatics pipeline, including the tools, NoDe and CATCh.

**Conclusions**

At the start of the FEMS-symposium 2015 the results and conclusions will be ready to be presented.



**FEMS-1878**

**Metagenomics in the environment**

**BIOINFORMATICS ALGORITHMS TO GET ACCURATE 16S RRNA HIGH THROUGHPUT SEQUENCING DATA.**

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<sup>2</sup>Bioscience Engineering, Vrije Universiteit Brussel, Brussels, Belgium

**Background**

The revolution in new sequencing technologies has led to an explosion of possible applications, including microbial biodiversity studies via bacterial 16S rRNA amplicon sequencing

**Objectives**

All 16S rRNA amplicon sequencing data suffer from the presence of erroneous sequences: (i) chimeric sequences introduced by the PCR reaction, and (ii) sequencing errors produced by the sequencing platform itself. As such, there is a need for efficient algorithms to remove those erroneous sequences in order to be able to accurately assess the microbial diversity.

**Methods**

First, a machine learning method called CATCh (Combining Algorithms to Track Chimeras) is able to integrate the output of existing chimera detection tools into a new more powerful method, outperforming all existing tools. Second, NoDe (Noise Detector) was introduced as an algorithm to correct 454 pyrosequencing errors, showing a significant improvement in reducing the error rate (reduction of 67 to 75%) and dramatic decrease in computational cost compared to state-of-the-art tools. Similarly, IPED (Illumina Paired End Denoiser) was introduced as first tool in the field for the denoising of MiSeq sequencing data.

**Conclusions**

Integrating both CATCh and NoDe/IPED into the preprocessing pipeline of 16S rRNA amplicon sequencing data significantly increases the reliability of the data, demonstrated by the positive effect on the clustering of the sequencing data in operational taxonomic units (OTUs). When tested on mock communities (MiSeq as well as 454 pyrosequencing platform), the number of OTUs closely approaches the actual number of species present in the mock samples. Both tools are freely available at <http://science.sckcen.be/en/Institutes/EHS/MCB/MIC/Bioinformatics/>.

**FEMS-3046**

**Metagenomics in the environment**

**MYCOSPHERE EFFECT OF THE PINE MUSHROOM (TRICHOLOMA MATSUTAKE) ON THE DIVERSITY, COMMUNITY STRUCTURE, AND FUNCTIONAL PROFILES OF SOIL MICROBES**

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<sup>1</sup>*School of Biological Sciences, Seoul National University, Seoul, Korea*

**Background**

The mycosphere is a distinct soil environment affected by fungal hyphal activities. Because mycosphere affects microbial activity, it is a valuable source of understanding microbial dynamics in soil ecosystem.

**Objectives**

The main objective of this study is to explore the mycosphere effect of *T. matsutake* by comparing bacterial and fungal diversity, community structure, and functional profiles in different zones of the fairy ring. We expanded on the design of past studies by comparing multiple fairy rings across two geographical locations.

**Methods**

We extracted soil DNA from mycosphere samples in fairy ring of *T. matsutake* and adjacent soil samples.

After processing pyrosequencing data, we conducted metagenomic analysis for microbial diversity, community structure, and functional profiles.

**Conclusions**

Across samples, community was similar within but different between mycosphere and non-mycosphere samples—microbial diversity was lower in the mycosphere compared to non-mycosphere zones. Many ectomycorrhizal fungi were negatively correlated with the presence of *T. matsutake*. Analysis of bacterial functional profiles predicted distinct bacterial activity between zones; in mycosphere samples, xenobiotic biodegradation and metabolism, and amino acid metabolism were high, but carbohydrate metabolism, and glycan biosynthesis and metabolism were low compared to non-mycosphere samples. Overall, similar functional activity of mycosphere zones across fairy rings and geographic locations imply a mycosphere effect of *T. matsutake*, by recruiting mutualistic taxa and excluding antagonistic ones based on the functional trait.

**FEMS-2666**

**Metagenomics in the environment**

**COMPARATIVE ANALYSIS OF METAGENOMES FROM FILTER UNITS OF FULL-SCALE HYBRID TREATMENT WETLAND**

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**Background**

Treatment wetlands (TW) have become widely used for wastewater treatment over the recent decade for their cost-effective building and maintenance. While physical and chemical pollutant removal mechanisms in TWs are well known, understanding of microbial processes related to pollutant removal in TWs is still low. Metagenomics offers the ability to examine directly the genomic content of microbial communities, complementing taxonomic information with functional capability.

**Objectives**

(1) to characterize microbial communities in vertical and horizontal sub-surface flow (VSSF and HSSF respectively) filter units of a full-scale TW treating wastewater from a schoolhouse for over ten years; (2) evaluate the functional potential of bacterial and archaeal communities.

**Methods**

Water samples, including influent, effluent and intermediate well, together with filter media biofilm from both units were used for shotgun sequencing with Illumina MiSeq system. Metagenomes were assembled into contigs and used for microbial profiling, identifying functional genes and relating them to nitrogen, phosphorus and carbon cycle.

**Conclusions**

Domain distributions showed the dominance of Bacteria (>95%), a small fraction of Eukaryotes (>2%), Archaea (>1%) and Viruses (0.2%). Samples of filter material had similar bacterial dominant phyla, but different distribution of lower taxonomic ranks. Wastewater samples had diverse bacterial community distribution between samples. Over one-third of archaeal sequences belonged to methanogenic species. Nitrogen metabolism in VSSF and HSSF filter units was dominated by ammonia assimilation related genes followed by nitrate and nitrite ammonification, denitrification and nitrogen fixation related genes. Phosphorous metabolism was dominated by phosphate metabolism genes.



## **FEMS-1561**

### **Metagenomics in the environment**

#### **HIERBIN - A METHOD FOR DETAILED FUNCTIONAL ANNOTATION AND QUANTIFICATION IN METAGENOMES**

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*University of Gothenburg and Chalmers University of Technology, Gothenburg, Sweden*

#### **Background**

Metagenomics aims for sequencing and identification of DNA from communities of microorganisms in their natural environment. By estimating and comparing gene abundances between samples we can get a picture of the functional role of the metagenome, i.e. which genes and pathways are abundant at different conditions. Methods for functional metagenomics typically rely on identification of functional domains such as PFAMs, TIGRFAMs and COGs. However, this results in a broad classification of gene products since many proteins can be classified into the same functional domain. The human gut gene catalog, for instance, is predicted to contain more than 5 million genes, but the number of PFAM domains is only around 10,000.

#### **Objectives**

We present HierBin, a new method for functional annotation and quantification in metagenomes, giving a more detailed functional description of the metagenome and performing better in identification of changes between conditions.

#### **Methods**

First a supervised classification step is used to identify known functional domains in the data. Secondly, each domain is divided into subdomains using unsupervised clustering based on sequence similarity. Finally the abundance of each subdomain is quantified by mapping the sequence reads to the subdomain sequence and differentially abundant subdomains are identified.

#### **Conclusions**

We show, by evaluating on resampled data, that our method performs better in predicting differential abundant genes than methods using only functional domains for gene prediction and detects differences at higher resolution that would be invisible at lower resolution.

Finally, we apply our method both to metagenomics data from the human gut as well as environmental samples.



**FEMS-2068**

**Metagenomics in the environment**

**METAGENOMIC ANALYSIS OF MICROBIAL COMMUNITIES IN RAPID SAND FILTER TREATING GROUNDWATER. COMMUNITY DIVERSITY AND METABOLIC POTENTIAL**

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<sup>2</sup>Center for Biological Sequence Analysis, Technical University of Denmark, Kgs. Lyngby, Denmark

**Background**

Rapid sand filtration is a drinking water production technology widely used in Europe and other countries around the world. This technique allows the removal of ammonia, manganese, ferrous iron, methane, sulfides and other compounds from groundwater by a combination of physical, chemical and biological processes. During many years, it has been recognized that microbial activity plays an important role in these processes.

**Objectives**

Therefore, it is essential to study the microbial composition in these rapid sand filters and how these communities interact and facilitate the removal of pollutants.

**Methods**

In this study, six samples from a rapid sand filter were analyzed through a metagenomic approach. By building a non-redundant gene catalog we identify the microbial diversity and the metabolic pathways occurring in this system.

**Conclusions**

*Nitrospirae* and *Proteobacteria* were the dominant phyla. The functional genetic potential included most of genes related to nitrogen oxidation (specially abundant in the nitrification pathway) and to methane oxidation. Genes involved in different CO<sub>2</sub> fixation pathways were also abundant. Genera typically implicated in manganese oxidation as *Hyphomicrobium* and in iron oxidation as *Gallionella* were also abundant in the catalog. This investigation has so far revealed the main biological processes occurring in these ecosystems. In the next step, we will try to assemble genomes of the most abundant genotypes of the rapid sand filter community.

**FEMS-2584**

## **Metagenomics in the environment**

### **INTRODUCING A COMPLETE METAGENOMICS ANALYSIS PIPELINE, BASED ON AN OPTIMAL COMBINATION OF ILLUMINA SEQUENCING TECHNOLOGY AND AN EASY TO USE ONLINE PLATFORM**

*W. Pirovano<sup>1</sup>, D. Derek Butler<sup>1</sup>, A. Costessi<sup>1</sup>*

<sup>1</sup>NGS, BaseClear, Leiden, Netherlands

#### **Background**

Direct sequencing of metagenomes is commonly implied for bacterial community analysis related to food research, biotechnological processes and clinical diagnostics.

#### **Objectives**

Commonly the taxonomic classification of sequences is based on unique target regions such as the 16S ribosomal gene which includes a number of unique variable regions. In this work we present an overview of a new and comprehensive Metagenomics analysis pipeline that we are using to prepare, sequence and analyze metagenomics samples from a range of environments.

#### **Methods**

In this holistic approach we start with laboratory techniques that take into account and reduce as much as possible the sources of possible bias that can be introduced into metagenomics datasets by laboratory steps like sampling procedures and DNA isolation. We further include both biological and technical controls to continually validate the process.

#### **Conclusions**

The bioinformatic analysis is performed using overlapping paired-end reads which are annotated through the Greengenes database (De Santis *et al.*, 2006). Finally we introduce the 'BaseClear metaGenome Browser' (BmGB)<sup>TM</sup>, <https://metagenomics.baseclear.com>, an online platform in which researchers (with or without any bioinformatics training) can easily interpret the taxonomic composition of hundreds of metagenomes using intuitive interfaces and efficient analyses. The workflow is tested on a number datasets and results are discussed.



**FEMS-1722**

**Metagenomics in the environment**

**LIMITS TO ROBUSTNESS, REPRODUCIBILITY AND ECOLOGICAL  
CONSISTENCY IN THE DEMARCATION OF OPERATIONAL TAXONOMIC UNITS**

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<sup>1</sup>*Institute for Molecular Life Sciences, University of Zurich, Zurich, Switzerland*

**Background**

The demarcation of *Operational Taxonomic Units* (OTUs) from complex sequencing datasets is a key step in large-scale characterizations of microbial communities. However, it remains unclear how well the various proposed OTU clustering algorithms approximate 'true' microbial taxa, and many conceptually distinct approaches continue to be used.

**Objectives**

We explored different aspects of OTU clustering, in particular the robustness, reproducibility and ecological consistency of OTU demarcation.

**Methods**

In a global and comprehensive survey of roughly one million microbial SSU sequences, we objectively quantified biases introduced by several widely employed algorithms.

**Conclusions**

We observed surprising trends in the robustness of sequence clustering to changing parameters, and with regard to the reproducibility of OTU-based findings. Moreover, we explored the ecological consistency of OTUs – based on the assumption that, like true microbial taxa, they should show measurable habitat preferences (niche conservatism). We systematically parsed sequence annotations to obtain broad ecological descriptions of sampling sites. Based on these, we observed that sequence-based microbial OTUs generally showed high levels of ecological consistency. However, different OTU clustering methods resulted in marked differences in the strength of this signal. We found that hierarchical average and complete linkage clustering provided the most robust and reproducible partitions with regard to a wide range of parameters. Moreover, complete linkage clustering provided the ecologically most consistent clusters. Assuming that ecological consistency can serve as an objective external benchmark for cluster quality, we

concluded that hierarchical complete linkage clustering, should be the default choice for OTU clustering.

**FEMS-2265**

**Metagenomics in the environment**

**MICROBIAL PROFILING OF TURFS ASSEMBLAGES FROM ABROLHOS BANK - BRAZIL, BAHIA**

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Background: Turf algae holobionts (host macroalgae plus microbiome) are widespread in coral reefs worldwide. They are also the most abundant benthic structures in the Abrolhos reef Bank (Brazil), with more than 50% benthic coverage. Abrolhos reefs turfs are morphologically diverse regarding color and texture, but their microbial matrix is not well known. Objectives: Our aim was to perform a metagenomic characterization of the prokaryotic microbiome of the different morphologic types of turf algae holobionts from Abrolhos reefs. Methods: We used metagenomic approaches to obtain the whole communities associated with turfs assemblages, collected at different reefs during March and October 2013, totaling 22 samples. Results: Filamentous, non-heterocystous cyanobacteria were the dominant microbial phototrophs of the holobionts. Main macroalgal in the holobiont were red, green and brown seaweeds. 15,27 million reads were generated using Illumina sequencing. Proteobacteria (36.8%, N = 1.564,678), Cyanobacteria (28.8%, N = 1.222,446), and Bacteroidetes (10.2%, N = 434,897 ) were dominant among the turf algae prokaryotic microbiome. Major microbial components of the turf algae holobiont are *Roseobacter* sp., *Silicibacter* sp., *Dinoroseobacter* sp., *Congregibacter* sp., *Maribacter* sp., and Cyanobacteria. Genes belonging to aerobic anoxygenic photosynthesis (AAnP), sulfur metabolism (e.g. S oxidation, and DMSP consumption) and virulence potential (adhesins, fibronectin/fibrinogen-binding protein, type IV pili, superoxide dismutase) were found in the turf algae microbiomes. Conclusion: The complex assemblies of microbial guilds render turf holobionts important competitive advantages allowing them to outcompete other benthic organisms and dominate vast reef areas.

**FEMS-1815**

**Metagenomics in the environment**

**EXPLORING MICROBIAL DIVERSITY PATTERNS IN THE SØR RONDANE MOUNTAINS (EAST ANTARCTICA) USING NEXT GENERATION SEQUENCING AND ARISA**

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<sup>3</sup>*Laboratory of Soil Science - Department of Geology and Soil Science, Ghent University, Ghent, Belgium*

**Background**

Antarctica is a continent of extremes. Low availability of liquid water and nutrients, extreme low temperatures and high levels of radiation exert high selective pressures on organisms. Consequently, most life forms are microbial. Estimations indicate that as little as 1-3 % of the continental surface represents ice-free regions. Relatively few biological studies focus on terrestrial samples which most often originate from the McMurdo dry valleys that make out the largest contiguous ice-free area. Mountain tops protruding through the ice sheets (*nunataks*) are less frequently studied although they might function as havens and reservoirs for terrestrial organisms. Geological data shows the presence of different kinds of bedrock in the Sør Rondane mountains (Queen Maud Land, East Antarctica). Patches of macroscopic organisms (lichens, mosses and arthropods) are scattered throughout these ice-free islands, indicating a high amount of variability in the presence of organic matter, and hence nutrients for microbial life. Here we present the results of a large scale sampling effort in the eastern Sør Rondane Mountains of such ice-free regions near the Belgian Princess Elisabeth station.

**Objectives**

We examined the composition and distribution of bacterial communities in these refugia, and investigated the possible impact of environmental parameters on these community compositions.

**Methods**

Samples were subjected to both a genetic fingerprinting technique (ARISA) and second generation sequencing (Illumina MiSeq 300PE).

**Conclusions**

Preliminary results indicate an effect of bedrock type and presence of macrobiotic organisms.

**FEMS-2075**

**Metals and microbes**

**CLONAL DYNAMICS OF CLINICALLY-RELEVANT MULTIDRUG RESISTANT SALMONELLA ENTERICA SEROTYPE 4,[5],12:i:- CIRCULATING IN EUROPE IN THE LAST DECADE**

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**Background**

*Salmonella* 4,[5],12:i:- has become a new epidemic serotype in Europe associated with human infections, being the identification/tracking of its clones crucial to contain their spread.

**Objectives**

We assessed the trends in *S.* 4,[5],12:i:- clones distribution and their association with antibiotic and metal resistance/tolerance genes in Portuguese isolates. Results were also compared with previous data from the last decade (2002-2010).

**Methods**

*S.* 4,[5],12:i:- isolates (n=158), confirmed by PCR (*fliB-fliA/fljB*), from different sources (clinical/food) and regions of Portugal (2010-2014) were analyzed. They were screened for sulfamethoxazole resistance genes (*sul1-sul2-sul3*) and other class 1 integrons genes (*int1*, *qac* and gene cassettes) by PCR. Detection of metal tolerance genes [copper (*pcoD*), silver/copper (*silA*) and mercury (*merA*)] and other antibiotic resistance genes by PCR, susceptibility to 10 antibiotics [ampicillin (A), chloramphenicol (C), gentamicin (G), kanamycin, nalidixic acid, ciprofloxacin, streptomycin (S), sulfamethoxazole (Su), tetracycline (T) and trimethoprim (Tr)] (CLSI/EUCAST) and clonality by PFGE were performed in representative isolates.

## Conclusions

We detected the presence of the 3 clones currently circulating in Europe: i) 'European clone' (75%; *sul2* and absence of *intl1/sul1/qacEdelta1*; mostly ASSuT-*bla*<sub>TEM</sub>-*strA-strB-sul2-tetB* and carrying *pcoD+silA+merA*), which has expanded throughout this study period; ii) 'Spanish clone' [6%; *intl1*; *qacEdelta1+qacH*; mostly AC(G)SSuTTr-*bla*<sub>TEM</sub>-*cmlA-floR-(aac(3)-IV)-aadA-sul1-sul2-sul3-tetA-dfrA12* and carrying *merA-silA*] and iii) 'Southern-European clone' (1%; *intl1*; *qacH*; CSSuTTr-*cmlA-aadA-strA-strB-sul3-tetB-dfrA12*) mostly with similar MDR and/or PFGE-types described since 2002. A marked decreased frequency of Spanish and Southern-European clones was observed contrasting with the expansion of the European clone characterized by ASSuT-phenotype and copper/silver/mercury tolerance genes, which might facilitate adaptation and success of these strains.

**FEMS-2396**

**Metals and microbes**

**BIOAUGMENTATION WITH METAL-RESISTANT PLANT GROWTH-PROMOTING RHIZOBACTERIA IMPROVE THE METAL RHIZOACCUMULATION POTENTIAL OF SPARTINA MARITIMA**

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**Background**

As an adjunct to phytoremediation strategies and as part of an effort to make this technology more efficacious, a number of scientists have begun to explore the possibility of using plants and the microbial populations colonizing the rhizosphere.

**Objectives**

A glasshouse experiment using soil from a metal-contaminated estuary was designed to investigate the effect of a native bacterial *consortium*, isolated from *Spartina maritima* rhizosphere and selected owing to their plant growth promoting (PGP) properties and multiresistance to heavy metals, in plant growth and metal accumulation.

**Methods**

Plants of *S. maritima* were randomly assigned to three soil bioaugmentation conditions (without inoculation, one inoculation and repeated inoculations during experimental period) for 30 days. Growth parameters and photosynthetic traits, together with total concentrations of several metals, including As, Cu, Pd and Zn were determined in roots and/or leaves.

**Conclusions**

Bacterial inoculation improved *S. maritima* root growth, through a beneficial effect on its photosynthetic rate ( $A_N$ ) due to its positive impact on functionality of PSII and chlorophyll concentration. Also, bacterial inoculation favoured water use efficiency (WUE) of *S. maritima*, through the increment in  $A_N$ , stomatal conductance and in root-to-shoot ratio. Moreover, this consortium was able to stimulate plant metal uptake specifically in roots, with increases of up to 19% for As, 65% for Cu, 40% for Pb and 29% for Zn. On the basis of these results, bioaugmentation of *S. maritima* with the selected bacterial consortium can be claimed to enhance plant adaptation and metal rhizoaccumulation during marsh restoration programs.

**FEMS-1911**

**Metals and microbes**

**ANALYSIS OF ADAPTATION TO HIGH CONCENTRATIONS OF NICKEL IONS OF A NOVEL SPHINGOBIUM STRAIN BY RNA-SEQ**

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**Background**

While bacteria belonging to the *Sphingobium* genus are generally known for their capacity to degrade poly-aromatic hydrocarbons, their response to high concentrations of metal ions has been largely disregarded. Only *S. cupriresistens*, a species isolated from a copper mine, has been reported to tolerate high concentrations (0.9 mM) of Cu<sup>2+</sup> [1]. We have recently reported the whole genome sequence of a novel *Sphingobium* strain able to tolerate Ni ions up to 20 mM [2].

**Objectives**

In order to understand the molecular mechanisms by which this strain is able to adapt to high concentrations of Ni ions we are currently investigating its transcriptome profiles by RNA-seq approach.

**Methods**

Sequencing of rRNA-depleted RNA was carried out for independent triplicate cultures in the presence or absence of 10 mM NiCl<sub>2</sub>, using the SureSelect strand-specific RNA library prep kit (Agilent) for Illumina MiSeq multiplex sequencing.

**Conclusions**

Transcriptomic data show the differential expression (fold change  $\geq |2|$ , *p*-adjusted < 0.05) of about one-hundred genes. Most of them are up-regulated in the presence of Ni<sup>2+</sup> and include genes for membrane proteins and metal efflux systems. Gene annotation was further enriched with description of operons and UTRs as identified by the EuGene integrative gene finder tool [3]. The observed metal resistance of this *Sphingobium* strain highlights its possible use for biodegradation of xenobiotic compounds in metal-rich environments.

1] Li *et al.*, 2013, Int J Syst Evol Microbiol, 63:604-609.

2] Manzari *et al.*, 2014, FEMS Microb. Letters, in press.

3] Sallet *et al.*, 2014, Bioinformatics, 30:2659-2661.





**FEMS-1586**

**Metals and microbes**

## **ADAPTABILITY OF TRICHODERMA ASPERELLUM TO HIGH CONCENTRATIONS OF VARIOUS METALS**

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### **Background**

*Trichoderma asperellum* is a biological agent capable of removing heavy metals from the environment via biosorption and/or bioaccumulation. Most studies investigated the use of dead fungal biomass for metal biosorption. The potentials of using live cells to transform toxic metals into less harmful forms (bioaccumulation) is lesser known.

### **Objectives**

In this study, we explored the adaptability of *T. asperellum* to increasing concentrations (100-6000 ppm) of various heavy metals (Al, Cd, Cr, Cu, Pb and Zn).

### **Methods**

Adaptive tolerance index (TI) of fungus in Potato Dextrose Agar was measured.

### **Conclusions**

The results conveyed bioaccumulation potential of *T. asperellum*. Results revealed that *T. asperellum* has adaptability towards increasing concentrations of Cd (TI: 0.01-0.04), Cr (TI: 0.22-0.28) and Cu (TI: 0.24-0.31) at the beginning of the experiment. Decreasing TI values (from 1.08 to 0.01) with increasing Al, Pb and Zn concentrations indicated poor adaptability. The most tolerable concentration across all metals, except Cu, was 1000 ppm. In fact, *T. asperellum* tolerated up to 6000 ppm Al. Concentrations of 1500-2000 ppm generally exerted inhibitory effects with Cu being most toxic (tolerable up to 300 ppm). The adaptability of *T. asperellum* to high metal concentrations was evident with SEM micrographs showing hyphae growth, in spite of some structural deformities, metal precipitates and particles deposited on the hyphal surface. Nevertheless, in cases where growth was inhibited, the severely deformed hyphae displayed irregular, grooves and rough texture. Our study highlights the potential of live *T. asperellum* cells in removing heavy metals from the environment in a cheap and environmental-friendly manner.

**FEMS-2077**

**Metals and microbes**

## **RECOVERY OF LITHIUM FOR NATIVE MICROORGANISMS FROM SALAR DE ATACAMA BRINES, CHILE.**

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### **Background**

Salar de Atacama (Chile) is the largest basin in Atacama Desert (2900 Km<sup>2</sup>) and is located at 2300 m.a.s.l. This hypersaline system possess an average concentration of lithium as brine of 1500 ppm. Lithium is obtained industrially through the process of solar evaporation ponds, reflecting the conditions present in this Salar, including high UV radiation, high evaporation and low rainfall; however, this process is non-selective and is pollutant. We propose the recovery of lithium from brines based on biosorption using Gram-positive bacteria selectively accumulate lithium

### **Objectives**

The aim of this study was to describe microbial diversity present in brines of Salar de Atacama and to isolate native bacteria with lithium adsorbent potential.

### **Methods**

Natural brines and concentrated lithium brines of Salar de Atacama were characterized. Such brines present hostile conditions for life, including high salt concentrations (0-100 g/L of Na<sup>+</sup>; 190- 450 g/L of Cl<sup>-</sup>; 0.2 -8 % of Li) and pH between 0.9 and 7. Simultaneously, microorganisms from the Salar were grown in a range of mineral mediums with or without organic carbon, and different concentrations of LiCl (0- 300 uM) through which the ability to absorb lithium was determined.

### **Conclusions**

Bacteria and Archaea were detected in both brines. These microorganisms are likely naturally adapted to the differences in concentration of the elements present, especially lithium. Furthermore, bacteria capable of adsorbing lithium after 12 hours were obtained. Analyses of microbial diversity of the brines and development of a process for obtaining lithium by native microorganisms are currently underway.



**FEMS-2009**

**Metals and microbes**

## **MODELING ARSENITE OXIDATION BY SINORHIZOBIUM SP. M14 IN CHANGING ENVIRONMENTAL CONDITIONS**

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### **Background**

A complement to the chemical methods of treatment of water contaminated with arsenic are the biological methods i.e. biooxidation. Among the biological methods, technologies using bacteria, which oxidize arsenites, appear to have significant potential. In the literature, many bacterial strains able to oxidize arsenates are described, but most of them are not able to survive in new conditions. These disadvantages can be overcome if we use strains or microflora enriched by the gene pool localized on the plasmid pSinA *Sinorhizobium* sp. M14, carrying genes responsible for arsenic metabolism.

### **Objectives**

The aims of this study were to (i) investigate the impact of biotic and abiotic factors, simulating the natural environment, on the growth kinetics and (ii) develop a kinetic model of arsenite oxidation by *Sinorhizobium* sp. M14.

### **Methods**

The effect of different factors on arsenite biooxidation (i.e. start OD, substrate concentration, mixture of arsenic compounds, temperature and pH) on the efficiency of oxidation of arsenites and the changes of physiological condition of the studied strains was investigated. Cultures were grown on MSM supplemented with 0,04% yeast extract. As speciation samples were analyzed by HPLC/ESI MS, while the amount of biomass was monitored by OD/CFU determination. Based on the collected data, a kinetic model of the process was developed.

### **Conclusions**

Culture of microorganisms in different concentrations of As (1;2,5;5 ppm), in mixture of arsenic compounds -As(III)/As(V) (2,5ppm/2,5ppm; 1ppm/4ppm), in different start OD (0,01;0,025;0,06;0,08;0,1;0,15), in various pH (4;6;7,5;10) and in different temperatures (10;16;22;30°C) allowed to develop a model of kinetics arsenite oxidation by *Sinorhizobium* sp. M14.

**FEMS-0914**

**Metals and microbes**

## **INVESTIGATIONS ON MULTIMETAL UPTAKE IN THE PRESENCE OF PESTICIDE BY FUNGAL ISOLATE**

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### **Background**

Wastewaters are often contaminated with complex cocktail of pollutants, out of which heavy metals and pesticides pose significant health risks. However, majority of the researches focus on bioremediation of individual metals or pesticides in isolation.

### **Objectives**

Therefore, efforts are needed to obtain microbial strains which can simultaneously handle multiple pollutants.

### **Methods**

Here a newly isolated fungal strain was assessed for its multimetal and pesticide uptake potential in the composite salts medium using glucose and lindane pesticide as the carbon and energy sources. This strain was able to grow in the presence of 1% glucose, 30 mg/L lindane and 30 mg/L multimetal (5 mg/L each of Cd<sup>2+</sup>, Cr<sup>6+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Pb<sup>2+</sup> and Zn<sup>2+</sup>) and could degrade maximum amount of lindane to 1.92 ± 0.01 mg/L in about 72 h. The cube root growth kinetic constant (k) of the strain was obtained as 0.0211 g<sup>1/3</sup> l<sup>-1/3</sup> h<sup>-1</sup> in batch study. However, the presence of lindane caused an inhibition effect in the uptake of heavy metals and thus only Pb and Zn were brought down below the permissible mandates for irrigation. The results indicate that the growth kinetics and pollutant uptake is affected by the nature of contaminant matrix. More studies towards the elucidation of mechanisms of metal removal by the fungus in presence and absence of pesticide by various spectroscopic and microscopic techniques such as SEM, TEM-EDX, FTIR, XRD and XPS have been conducted.

### **Conclusions**

These results reveal the inherent potential of this fungal strain to remove multimetals in the presence of pesticide from contaminated wastewater.

**FEMS-3001**

**Metals and microbes**

**SEARCH FOR BACTERIA IN A MEDIEVAL ARCHEOLOGICAL SITE IN VERDUN (FRANCE) AND ISOLATION OF CUPRIAVIDUS NECATOR B9, A NEW METAL RESISTANT STRAIN**

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**Background**

Remains of a well-preserved medieval foundry were excavated by archaeologists in 2013 in Verdun (France). Three to four ancient workshops specialized in brass and copper alloys were found with an activity between 13<sup>th</sup> to 16<sup>th</sup> c. Levels of Cu, Zn and Pb reached 20 000, 7000 and 6000 mg kg<sup>-1</sup> (dw), respectively, in several soil horizons.

**Objectives**

The objective was to examine the microbial community in this contaminated site.

**Methods**

A total of 8–22 10<sup>6</sup> reads were obtained by shotgun metagenomics in each horizon (length : ± 220 pb). Bioinformatic analyses suggest the presence of very complex bacterial communities dominated by Proteobacteria. Using selective media three *Cupriavidus necator/eutrophus* strains were isolated. One of those strains, strain B9, differed from *C. necator* N1 and *C.eutrophus* H16 on several aspects. For instance, strain B9 was not able of autotrophic growth but was copper resistant (up to 1.2 mM). The strain features a plasmid that differs in size in comparison to the one observed in *R. eutropha* H16. Strain B9 was therefore characterized through genomic sequencing and a total of 516 contigs were obtained (Illumina sequencing, N50=25246 pb).

**Conclusions**

Preliminary analyses of the contigs confirm the absence of *cbb* genes necessary for autotrophy and show the presence of a ~70kb region containing metal detoxication genes (mainly copper but also a mercury resistance *merRTPCA* operon) also found in environmental *Ralstonia pickettii* strains and mainly an extended cluster of *cop* genes (*cop KH3SRABCDIH2JGFOL1L2QH*) largely syntenic with the *cop* region of *C. metallidurans* plasmid pMOL30.

**FEMS-3034**

**Metals and microbes**

## **METAGENOMIC PROFILING OF MICROBIAL METAL INTERACTION IN RED SEA DEEP-ANOXIC BRINE POOLS' WATER**

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### **Background**

Different geochemical studies reported high metal abundance in Red Sea deep-anoxic brine pools, especially in Atlantis II Deep, which has the highest metals content. Brine pools showed wide diversity of biologically essential and non-essential metals. Several metals known for their toxicity to biological life were detected in these pools. Yet, our previous microbiome analyses of the pools demonstrated vast microbiological diversity.

### **Objectives**

In this study, we compare metal-resistant prokaryotic microbiomes in different metal-rich brine water samples from; Atlantis II lower convective layer (ATII-LCL), Atlantis II upper convective layer (ATII-UCL), Discovery Deep (DD) and Kebrit Deep (KD). Moreover, we investigate genome evolution of microbial communities in response to such excessive metal abundance.

### **Methods**

Using 16S rRNA pyrotags and shot-gun 454-pyrosequencing, we perform a comparative analyses of a-taxonomic assignment of Operational Taxonomic Units to major bacterial and archaeal groups and b-metal resistant protein-coding genes, of the microbial communities and metagenomes. The ATII-LCL, ATII-UCL, DD and KD brine pools metagenomes protein-coding genes involved in microbial-metal interaction and resistances were assessed for abundance, diversity and novelty.

### **Conclusions**

We report specific microbial diversity of these three brine pools. Functional analyses of the metagenomes revealed different metal resistance mechanisms. This was supported by the strong correlation between specific high metal/s concentration in selected brine water, where; metal resistance, enrichment of metals metabolism and transport were revealed. As expected, ATII-LCL showed the highest relative abundance of genes involved in microbial-metal interaction. Additionally, we report significant abundance of peroxidases-encoding genes, mainly in ATII-LCL, and we hypothesize that generation of H<sub>2</sub>O<sub>2</sub> occurs through interaction of pyrite deposits.



## **FEMS-3104**

### **Metals and microbes**

#### **CATION METABOLISM AND TRANSLOCATION IN BROWN ROT WOOD DEGRADING FUNGI**

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#### **Background**

The brown rot fungi are important components of the coniferous northern hemisphere forest ecosystem and are characterized by their ability to colonize and degrade lignocellulosic materials; preferentially metabolizing hemicellulose and cellulose components and leaving a residue of modified lignin. Multiple aspects of the metabolism and degradative capabilities of these organisms are dependent upon cations, including but not limited to iron, manganese, and calcium.

#### **Objectives**

As wood degrades, it is characterized by decreasing pH and increasing concentrations of selected cations. The decay fungi, as they colonize wood, differentially translocate cations and actively modulate their ionic environment. The base cations are needed for many metabolic functions including membrane stabilization and they also function as enzyme cofactors and electrolytes. Transition metals are directly involved in lignocellulose degradation. We will examine the influence of cation concentrations on fungal sheath formation, and the activity of a biologically unique iron-based non-enzymatic degradative system in the brown rots.

#### **Methods**

The role of iron in the chelator-mediated Fenton (CMF) system will be examined as it is particularly crucial to the ability of the brown rot fungi to depolymerize lignocellulose. Methods used include but are not limited to ICP and XFM. Organisms used in this work include: *Serpula lacrymans*, *Postia placenta*, *Fomitopsis pinicola* and *Gloeophyllum trabeum*.

#### **Conclusions**

The role played by transition metals, pH, oxalate production and calcium oxalate crystal formation in brown rot physiology and cell wall breakdown mechanisms is discussed along with the potential ecological significance of cation mobilization and redistribution within the forest floor ecosystem.

**FEMS-1474**

**Metals and microbes**

**ADHESIN COMPETENCE REPRESSOR (ADCR) FROM STREPTOCOCCUS PYOGENES CONTROLS ADAPTIVE RESPONSES TO ZINC LIMITATION AND CONTRIBUTES TO VIRULENCE**

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**Background**

Altering zinc bioavailability to bacterial pathogens is a key component of host innate immunity. Thus, the ability to sense and adapt to the alterations in zinc concentrations is critical for bacterial survival and pathogenesis.

**Objectives**

To understand the adaptive responses of group A *Streptococcus* (GAS) to zinc limitation

To elucidate the regulation of adaptive responses by metalloregulator AdcR

**Methods**

RNA sequencing

Gel mobility shift assay

qRT-PCR

animal infection studies

X-ray crystallography

**Conclusions**

AdcR regulates the expression of 70 genes involved in zinc acquisition and virulence. Zinc-bound AdcR interacts with operator sequences in the negatively regulated promoters and mediates differential regulation of target genes in response to zinc deficiency.

Genes involved in zinc mobilization and conservation are derepressed during mild zinc deficiency, whereas the energy-dependent zinc importers are upregulated during severe zinc deficiency.

We also demonstrated that transcription activation by AdcR occurs by direct binding to the promoter. However, the repression and activation by AdcR is mediated by its interactions with two distinct operator sequences.

Mutational analysis of the metal ligands of AdcR caused impaired DNA binding and attenuated virulence, indicating that zinc sensing by AdcR is critical for GAS pathogenesis. Together, we demonstrate that AdcR regulates GAS adaptive responses to zinc limitation and identify molecular components required for GAS survival during zinc deficiency.

**FEMS-2759**

**Metals and microbes**

**MECHANISMS OF CHROMATE RESISTANCE AND REDUCTION BY  
KLEBSIELLA PNEUMONIAE CHROAq1 ISOLATED FROM A CHROMIUM  
CONTAMINATED AQUIFER**

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**Background**

Chromium-reducing microorganisms as well as their enzymes chromate-reductases have taken great interest due to their potential application in bioremediation processes. They are phylogenetic and metabolically diverse; in some cases Cr(VI) reduction is result of the metal respiration as in *Geobacter*. However in other microorganisms like genus *Klebsiella* has little knowledge about the metal reduction mechanisms.

**Objectives**

The present study was aimed at the characterization the mechanisms of Cr(VI) resistance and reduction in *Klebsiella pneumoniae* ChroAq1, isolated from long-term Cr(VI)-contaminated aquifer located in Guanajuato, México.

**Methods**

It was determined the ability to reduce Cr(VI) to Cr(III) by growing cells and cell-free extracts of *K. pneumoniae* ChroAq1. The cellular fractionation was performed by ultracentrifugation. Moreover the complete genome of ChroAq1 was sequenced with illumina technology, automatically annotated and analyzed. Some putative genes involved in resistance and reduction were deleted by **double recombination**.

**Conclusions**

The isolated strain ChroAq1 was identified as *Klebsiella pneumoniae*, its resistance observed was 1.8mM of Cr(VI) in anaerobic conditions and 22mM in aerobic conditions and was able to reduce Cr(VI) only anaerobically. The cell-free extract from this strain showed a NAD(P)H-dependent chromate-reductase activity associated to the soluble cell fraction. The gen *chrA* is present in the genome of

ChroAq1 however its deletion did not abolish the chromate resistance. In other hand, we deleted four genes presumptively involved in Cr(VI) reduction, one encodes a protein 84.57% identical to YieF (ABJ74146), other encodes one 86.58% identical to NemA (P77258) from *E. coli*, however they had no effect on the chromate reduction capability, the other two are being studied.

**FEMS-1598**

**Metals and microbes**

**PRODUCTION OF SELENIUM AND ZINC ENRICHED BIOMASS AND NANOPARTICLES USING LACTOBACILLI**

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**Background**

Selenium (Se) and Zinc (Zn), are the trace elements which play a pivotal role in humans well-being. Symptoms resulting from deficiency of these essential metals are diverse but chronic and severe and if untreated, can prove fatal. Bioproduction of Se and Zn enriched biomass and nanoparticles by lactobacilli can be explored and exploited for development of dietary organic sources of these minerals to overcome the mineral deficiencies and to improve the human health.

**Objectives**

To produce Se and Zn enriched biomass and Se<sup>0</sup> and zinc oxide nanoparticles using lactobacillus spp.

**Methods**

Fifty human origin lactobacilli were screened for their ability to accumulate Se and Zn by growing them on medium added with different concentration of sodium selenite and zinc sulfate respectively. Estimation of Se and Zn accumulated by cultures was carried out by ICPES and AAS, respectively. Selected cultures were used for biogenic production of Se and ZnO nanoparticles and their size were determined by Scanning Electron Microscope.

**Conclusions**

Among all the isolates, *L. reuteri* NCDC77 was found to have greater ability to accumulate Se (820 µg/g dry weight) coupled with potential probiotic properties while *L. plantarum* YM2C was able to accumulate large amount of Zn (1.32mg/g dry weight). In addition, nanoparticles of Se and Zn produced by the selected strains, were found to be in the range of 100-200nm. Harnessing these potential cultures can serve as a novel application of these cell factories for the production of Se and Zn enriched functional foods and as dietary supplement.

**FEMS-1403**

**Metals and microbes**

**ACCURATE MINIMAL INHIBITORY CONCENTRATION PREDICTION FOR CUPRIAVIDUS METALLIDURANS CH34 USING A TOXICITY MODEL BASED ON TWO CHEMICAL METAL-ION CHARACTERISTICS**

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**Background**

The inhibitory effect of metal(-ion)s on the growth of microorganisms is mainly determined by their chemical binding properties to essential biochemical molecules (e.g. thiols, proteins, enzymes, RNAs, DNA) and the effective intracellular concentration. Active control of the intracellular metal-ion concentration is one of the successful survival strategies microorganisms use against toxic metal-ion concentrations and they can harbor a variety of resistance systems to control the intracellular concentration of different metal-ions. *Cupriavidus metallidurans* CH34 is one of the model bacteria used to study survival in the presence of elevated metal-ion concentrations. Numerous studies have been performed using *C. metallidurans* CH34 to elucidate its various metal-ion defense mechanisms. As a consequence, a substantial amount of physical, chemical and biological data is available on the impact of several metal-ions on the behavior of *C. metallidurans* CH34.

**Objectives**

To test whether it is possible to establish a metal-ion toxicity model that is able to predict the Minimal Inhibitory Concentration for *C. metallidurans* CH34.

**Methods**

A two-variable metal-ion characteristics toxicity model, based on  $|\log K_{OH}|$  ( $K_{OH}$  = first hydrolysis constant) and  $\chi^2_{m.r}$  (= covalent index), that efficiently correlates ( $r^2=0.90$ ) with the measured MIC of 20 different metal ions, has been developed for *C. metallidurans* CH34 cultivated in chelating-free mineral medium. To test this chemical model for its applicability, the MIC of  $Pd^{2+}$  was first calculated and afterwards experimentally determined.

**Conclusions**

The model prediction, 0.032 mM, corresponded very well to the measured MIC of 0.029 mM, indicating that the two metal-ion characteristics,  $K_{OH}$  and  $\chi^2_{m.r}$ , can be used to reliably predict MIC values.

**FEMS-2221**

**Metals and microbes**

**SEVEN MERA ALLELES ARE SPREAD AMONG ENTEROCOCCUS FROM  
DIFERENT SPECIES, ENVIRONMENTS AND CLONAL BACKGROUNDS (1926-  
2012)**

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**Background**

Successful bacteria accumulated different genetic features shaping their evolution and fitness to diverse environments/hosts. Mercury (Hg) is widespread in Nature and possibly contributed for selection of particular strains.

**Objectives**

To evaluate the dispersion of diverse *merA* alleles among enterococci from several origins and clonal backgrounds.

**Methods**

*merA* alleles was searched in *Enterococcus* available genomes, used to construct a maximum-likelihood phylogenetic tree. A PCR scheme+RFLP+sequencing was developed to detect the six *merA* alleles identified, namely I-(GenBank-AECE01000068), IIA-(AIUL01000023.1), IIB-(ASDU01000008.1), III-(NZ\_KB947199.1), IV-(AECE01000068.1) and VI-(NZ\_KB030055.1), among 918 *Enterococcus* (Portugal; human/animal/environment/food; 1996-2012). Clonality was evaluated by PFGE/MLST, *merA*-transfer by conjugation and Hg-phenotype in m\_Enterococcus+128mg/L HgCl<sub>2</sub>.

**Conclusions**

*merA* were found in 4% (n=37/918) of *Enterococcus* studied. PCR+RFLP+sequencing distinguished the six alleles previously found at Genbank and a new one (type V). They were distributed among diverse sources and clones, in our and GenBank isolates: types I and IV-human *E. faecalis* (*Efls*; n=5; USA; 1987-88; ST9/ST206); IIA-human/pig/trout/feed/hospital-sewage *E. faecium*-

*Efm/E.hirae/Enterococcus\_sp* (n=17/1/1; Portugal/France/Denmark/Germany; 1989-2012; 12ST-*Efm* including from ST78-ST17-ST18-lineages-CC17); IIB-human *Efls/E.dispar* (n=2/1; Portugal/USA; 1926-2001; ST206/ST105/ST107); III-human/animal/hospital-sewage *Efls/E.casseliflavus* (n=15/1; Portugal/France/USA/Canada/Japan; 1961-2012; ST64/ST9/ST30/ST159/ST245); V-human/trout/pig-manure *Efm* (n=4; Portugal; 2001-2006; ST94/ST890); VI-human/hospital-sewage/piggery-soil/piggery-manure/trout *Efm* (n=23; Portugal, Italy France, Germany, Denmark, Hungary, Switzerland, Ireland, Norway, Brazil, USA; 1961-2012; 14ST including ST78-ST17-ST18-lineages-CC17). The presence of *merA* among major *Efm/ST9-Efls* lineages associated with human infections, suggests that mercurial-compounds potentially contributed for their selection/maintenance. *merA*<sup>+</sup>-*Enterococcus* grown in >128mg/L-HgCl<sub>2</sub>. Transfer occurred for IIA and VI alleles. All but VI-*merA* alleles, were also identified in genomes of different species of Firmicutes (data not shown), suggesting genetic exchange of enterococci with bacteria sharing the same communities/environmental challenges.



**FEMS-1348**

**Metals and microbes**

**THE SENSORY PROTEIN HBPS FROM THE SOIL BACTERIUM STREPTOMYCES RETICULI SPECIFICALLY INTERACTS WITH IRON, HEME AND AQUO-COBALAMIN**

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**Background**

A novel type of redox-signaling system has been recently characterised in bacteria, the HbpS-SenS-SenR system from streptomycetes. The extracellular HbpS protein binds ferrous ions, heme and aquo-cobalamin.

**Objectives**

Elucidation of molecular mechanisms that modulate the function of HbpS-SenS-SenR, and analyses of binding sites as well as binding kinetics in HbpS-ligand interactions.

**Methods**

Disruption and site-directed mutagenesis; growth assays; crystallization; FRET, UV/Vis and CD spectroscopy; bioinformatics

**Conclusions**

HbpS-SenS-SenR is involved in the protection of *Streptomyces* against oxidative stress and is conserved in many other actinobacteria. HbpS acts as an accessory module of the two-component system SenS-SenR. Analysis of the HbpS crystal structure and biochemical studies revealed that HbpS assembles as an octamer that is crucial for interaction with the sensor kinase SenS. Under conditions of oxidative stress this leads to the autophosphorylation of SenS that, in turn, phosphorylates the response regulator SenR. This activates the transcription of anti-oxidative genes.

HbpS sequesters large quantities of ferrous iron ions which might protect *Streptomyces* from the effects of iron-based oxidative stress. HbpS is also an unusual heme-binding protein in which a specific threonine apparently binds to the tetrapyrrole macrocycle. *In vitro* and *in vivo* studies have also shown that HbpS can degrade heme. This activity may be responsible for HbpS-mediated protection against toxic concentrations of heme. Moreover, HbpS binds aquo-cobalamin. The calculated  $K_d$  of 34  $\mu$ M suggests that HbpS might bind aquo-cobalamin in both bacterial cultures and in the *Streptomyces* natural environment the soil. The physiological relevance of this interaction remains, however, to be elucidated.

**FEMS-2730**

**Metals and microbes**

## **CALCIUM CARBONATE MINERALIZATION IN BACILLUS SUBTILIS: STUDIES AND APPLICATIONS**

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### **Background**

Calcium carbonate precipitation is a widespread phenomenon among bacteria, with relevant implications in natural processes and great potentiality in numerous applications. Nevertheless, the molecular aspects of the process are still unknown.

### **Objectives**

We have been studying CaCO<sub>3</sub> precipitation in the model bacterium *Bacillus subtilis* by different multidisciplinary approaches to identify: a) bacterial genes, and b) cell structure(s), involved in biomineralization; c) analytical markers able to distinguish the "biosignature" of natural calcite.

### **Methods**

For objective a), we produced *B. subtilis* mutants by insertional mutagenesis and checked their precipitation phenotype. For objective b), we tested bacterial dead cells and cell fractions in a suitable precipitation test in solution. For objective c), we analyzed calcite crystals produced by *B. subtilis* by X-ray powder diffraction (XRPD) and Electron Paramagnetic Resonance (EPR) spectroscopy.

### **Conclusions**

By analysis of mutants impaired in calcite formation, we identified a gene cluster as involved in the process, and two genes of the cluster, *etfA* and *etfB*, as the minimal gene set necessary for precipitation.

The *B. subtilis* cell wall fraction, called BCF, was able to induce calcite formation in solution. BCF was applied on stone specimens and on a monumental site as an eco-friendly biotreatment for stone consolidation. New calcite formation and a little cohesion increase were observed in the treated stones.

Both XRPD and EPR revealed unusual spectral parameters, attributed to the effects of the bioprecipitation of the mineral. The resultant spectroscopic fingerprint of bacterial calcite would be useful for identifying traces of bacterial activity in fossil carbonate deposits.



**FEMS-1149**

**Metals and microbes**

### **ALUMINUM TOXICITY AND TOLERANCE IN MICROORGANISMS**

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#### **Background**

In acid soils below pH 4.5, aluminum becomes more soluble and toxic to plants, a few micronutrients, such as manganese and iron, become more soluble and toxic; and most plant nutrients, such as phosphorus, become more limited. This situation can cause serious destruction of the environment and significant economic problems. To overcome these problems, understanding of the toxicity of acid soils and the tolerance mechanisms in plants and microorganisms, especially the Al-toxicity and tolerance mechanisms, is essential.

#### **Objectives**

We have isolated many plants and microorganisms well adapted to highly acidic soils. Although there have been numerous reports on molecular mechanisms of Al tolerance in plants and microorganisms, such as organic acid secretion, alteration of cell walls, and Al-sequestration, our isolates suggested the complexity of the mechanisms of Al toxicity and tolerance. In this study we studied the Al-toxicity and tolerant mechanisms.

#### **Methods**

Microorganisms: *Acidocella aluminidurans* NBRC 104303(T), *Burkholderia bannensis* NBRC 103871(T), *B. acidipaludis* NBRC 101816(T) and 103872, *B. helei* NBRC 101817(T), *Pullulanibacillus* sp. CA42, *Rhodotorula taiwanensis* CGMCC 2.4753, and *Saccharomyces cerevisiae* BY4741 and their derivatives.

Al tolerance test: Low phosphate synthetic minimal medium containing 1% glucose was buffered with 5 M succinic acid at pH 4.5. Cells were cultured in the presence of different concentrations Al at 28°C.

#### **Conclusions**

The results suggest that in addition to the aforementioned mechanisms of Al tolerance, an extracellular polysaccharide, cellular functions, and alteration of

membrane lipids, are relevant with the Al toxicity and tolerance. The knowledge will greatly help to overcome the acid soil problems.

**FEMS-1645**

**Metals and microbes**

**REDUCTION OF CHALCOGEN OXYANIONS AND BIOGENESIS OF NANOPARTICLES BY *OCHROBACTRUM* SP. MPV1**

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**Background**

Metal nanoparticles have been produced using chemical and physical methods for many years. However, the exploitation of strong reducing agents may lead to undesired toxicity issues. It is, therefore, important to develop alternative and ecofriendly methods. Recently biosynthetic methods employing microorganisms have emerged as simple and viable alternative to produce metal/metalloid nanoparticles. In particular, the chalcogens selenium and tellurium in their elemental forms exhibited interesting antimicrobial activity. Moreover, nanoparticles produced with these elements show intriguing optoelectronic and semiconducting properties.

**Objectives**

A microbial strain characterized as *Ochrobactrum* sp. MPV1, isolated from the highly metal-polluted site of Scarlino (GR), was studied for its ability of effectively reducing both the oxyanions selenite and tellurite to their elemental forms in aerobic conditions and consequently producing elemental selenium and tellurium nanoparticles.

**Methods**

The isolate is capable of reducing 2 mM  $\text{SeO}_3^{2-}$  in 48 hours and 0.3 mM  $\text{TeO}_3^{2-}$  in 96 hours. The intracellular accumulation of nanoprecipitates was demonstrated through SEM-EDX and TEM analysis. Moreover, several analysis were performed in order to shed light on the mechanisms involved in selenite and tellurite reduction to the elemental state. The results obtained suggest that selenite and tellurite are reduced through two different mechanisms in *Ochrobactrum* sp. MPV1. Glutathione seems to play a major role in selenite reduction, while tellurite reduction could be ascribed to the catalytic activity of intracellular NADH-dependent oxidoreductases.

**Conclusions**

In conclusion, *Ochrobactrum* sp. MPV1 is an ideal candidate for the biogenesis of  $\text{Se}_0$  and  $\text{Te}_0$  nanoparticles, with possible biotechnological and industrial applications.

**FEMS-2302**

**Microbes and alternative energy sources**

**INVESTIGATION OF FACTORS DETERMINING STRESS RESISTANCE OF SOLVENTOGENIC BACTERIAE STRAIN CLOSTRIDIUM ACETOBUTYLICUM DERIVED BY ADAPTIVE SELECTION METHOD**

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**Background**

Biobutanol is a promising type of motor fuel and feedstock for chemical industry.

**Objectives**

To elaborate efficient technology of biobutanol manufacturing from renewable materials adaptive selection technique was applied to derive solventogenic bacterial strain *Clostridium acetobutylicum* producing butanol in concentration up to 18.7 g/l. It was shown that the selected bacterial strain when compared to the parent strain was capable to withstand 1.3 times higher butanol level and was distinguished by osmotolerance, resistance to temperature impact and antibiotic chloramphenicol.

**Methods**

To define the factors governing enhanced resistance of selected strain *C. acetobutylicum* to stress exposure, lipid fatty acid composition of cellular membranes in the parent and butanol-adapted clostridial strains was analyzed. Using real-time PCR technique, we carried out comparative examination of expression of heat shock gene *groEL* and key gene *adhE* responsible for butanol biosynthesis in parent and adapted clostridial strains grown on butanol and solvent-free media.

**Conclusions**

It was found that the ratio of saturated to unsaturated acids is 1.84 times higher in butanol adapted strain *C. acetobutylicum* than in parent strain, which leads to stabilization of structure, promotes membrane fluidity and induces resistance to stress factors.

It was established that expression of *groEL* gene in the parent strain declined 100-fold on butanol-containing media, while expression of gene *adhE* was not recorded, in contrast to adapted strain *C. acetobutylicum* showing only minor decrease of gene expression under similar conditions. The obtained results indicate that cell metabolism of adapted bacterial strain was less sensitive to adverse butanol effects as compared to wild-type bacteria.





**FEMS-2004**

**Microbes and alternative energy sources**

### **METHANOGENIC CONSORTIA STRUCTURE AND STABILITY CHANGES AFTER LONG-TERM STORAGE**

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#### **Background**

The start-up of trial operation of biogas plant usually consists of adding leachate from other biogas plants or inoculation by cattle slurry. An alternative to the traditional start-up operation is the use of the freeze-dried microbial consortia that were previously selected in anaerobic digestion process.

#### **Objectives**

The aims of this work were investigate the effects of freeze-drying of methanogenic consortium selected from agriculture anaerobic digestion plant on: (i) biogas production efficiency and (ii) stability of microbial community structure.

#### **Methods**

Lab-scale anaerobic digestion process. Freeze-drying. Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), ammonium. Determination of total cell counts by fluorescence microscope (Japan) using the filter set WU for DAPI detection. DNA and RNA isolation. PCR amplification and 16S rRNA gene amplicons libraries.

#### **Conclusions**

The conducted analyses revealed that the consortium selected from anaerobic digester plant is active and able to effectively produce biogas after long-term storage. 16S rRNA gene amplicon libraries analyses showed that the microbial consortia have the same methanogenic community composition after long-term storage. These results demonstrated that described method for long-term storage and revitalization of methanogenic consortia could be used as a starter to quick start-up industrial process of biogas production.

**FEMS-1452**

**Microbes and alternative energy sources**

## **GENETIC ENGINEERING OF CYANOBACTERIA FOR HYDROGEN PRODUCTION**

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### **Background**

Hydrogen can be naturally produced by several groups of organisms, including cyanobacteria. As a biofuel, hydrogen has substantial advantages over carbon-based compounds as it does not generate carbon dioxide and other atmospheric pollutants, and has a higher mass energy density than any other fuel. However, the current cost of its industrial production and the low yield of hydrogen by living organisms are not conducive to increasing its use as an environmentally friendly alternative to the traditional fuels.

### **Objectives**

Our goal was genetic engineering of cyanobacteria for the purpose of increasing the yield of hydrogen that was generated by these strains.

### **Methods**

For this purpose, we employed homologous and heterologous expression of hydrogenases in cyanobacteria.

### **Conclusions**

Genetic engineering of cyanobacteria allowed us to significantly increase their hydrogen-producing capacity without compromising the stability of the mutant strains. As the details of molecular regulation of hydrogen production in the cell become increasingly available, new steps are being taken to improve the level of hydrogen production by cyanobacteria. Due to a photoautotrophic nature and low maintenance cost of these microorganisms, they represent the most economical system for biological hydrogen production with a potential for commercial application.

**FEMS-0752**

**Microbes and alternative energy sources**

**NEW GENETIC APPROACH TO ENGINEERING OF MUTANT STRAINS OF  
CYANOBACTERIUM ANABAENA VARIABILIS ATCC 29413 WITH ENHANCED  
LEVELS OF BIOHYDROGEN PHOTOPRODUCTION**

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**Background**

Heterocystous cyanobacteria are capable to efficient nitrogenase-dependent H<sub>2</sub>-photoproduction in the case of mutational elimination of uptake hydrogenase activity. In this study we used previously isolated chemically induced H<sub>2</sub>-producing mutants of *A. variabilis* PK84 and PK17 with impaired activity of uptake hydrogenase and also ammonium producing ethylenediamine-resistant (ER<sup>R</sup>) mutants ED21 and ED93 characterized by derepressed levels of nitrogenase activity and low levels of glutamine synthetase activity. Our sequencing data revealed the point mutations in conservative sites of genes *hypF* (PK84: Asp374Asn), *hupL* (PK17: Cys446Tyr), *glnA* (ED21: Ala382Thr, ED93: Pro465Leu).

**Objectives**

The construction of *A. variabilis* strains with mutations in both *hypF* (or *hupL*) and *glnA* genes and analysis of their H<sub>2</sub>-producing capacities in comparison with reference strains PK84 and PK17.

**Methods**

The mobilizable plasmid vector pRL498 was used for cloning and transfer of DNA fragments with mutations into *Anabaena* cells via triparental mating with *E. coli*. Mutant strains PK84 and PK17 were used as recipients for cloned fragments with *glnA*-mutations (with selection of ED<sup>R</sup> recombinants) whereas mutants ED21 and ED93 were recipients for *hypF* or *hupL* genes inactivated by insertion of spectinomycin resistance cassette (with selection of Sp<sup>R</sup> recombinants)

**Conclusions**

In both variants of gene transfer we have selected some biotechnologically perspective double mutants with lower growth rate and higher level of H<sub>2</sub>-production compared with strains PK84 and PK17 (up to 130 % per biomass unit) that might be explained by enhanced levels of nitrogenase activity accompanying by a partial block in biosynthetic or assimilatory processes caused by additional alterations in *glnA* gene.

**FEMS-2771**

**Microbes and alternative energy sources**

## **IMMOBILIZED BIOCATALYSTS FOR BIODIESEL FUEL PRODUCTION**

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### **Background**

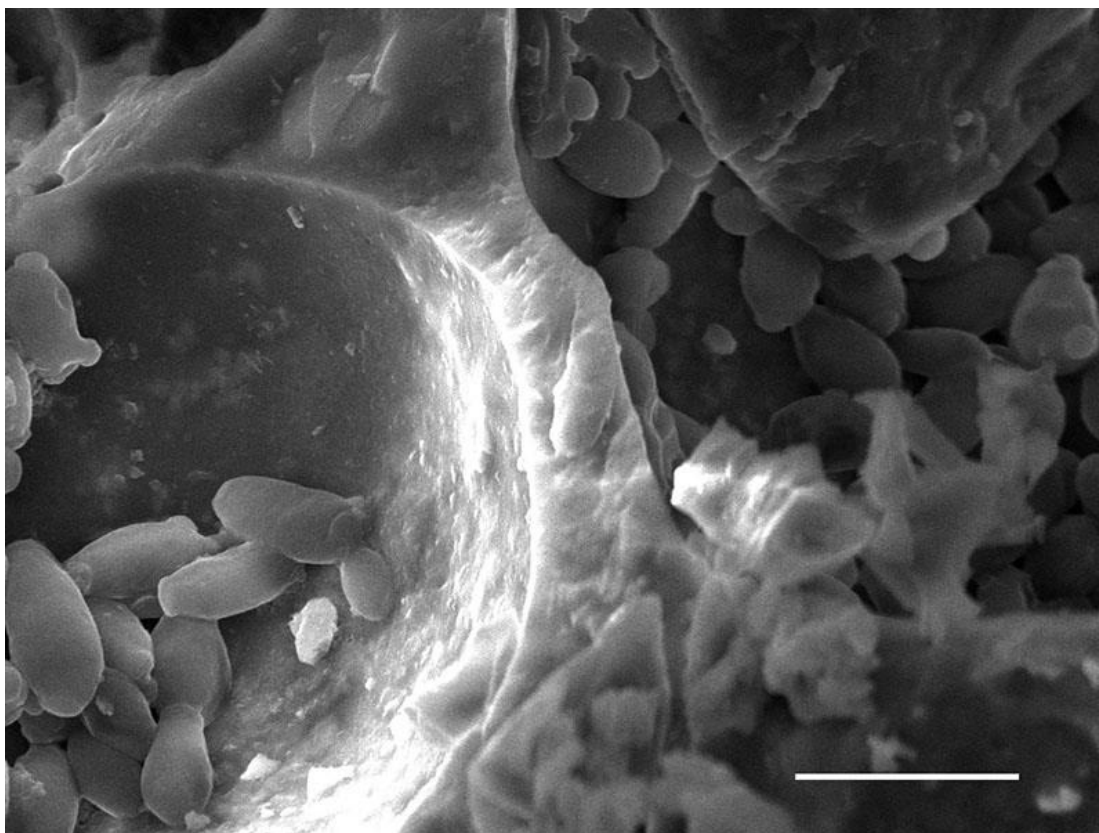
Biodiesel comprising of fatty acid alkyl esters is a promising alternative fuel. The biodiesel fuel can be produced under milder conditions and with fewer steps using biocatalysts instead of conventional alkali catalyst. In order to increase the biocatalyst stability and to ensure its easy separation from the biodiesel fuel, it is required to immobilize the biocatalyst on a suitable carrier. The comparison of biodiesel fuel production with different immobilized biocatalysts will be helpful for the process optimization and for further industrial implementation.

### **Objectives**

In our work, we studied the immobilization of lipases on silica, diatomite, and ion exchange resins, and the immobilization of yeast cells and mycelium on both soft (polyurethane foam) and rigid (haydite) carriers to perform the comparison of different immobilized biocatalysts in stirred-tank and packed bed bioreactors.

### **Methods**

*Pseudomonas fluorescens* lipase, *Rhizopus oryzae* lipase, Lipase B *Candida antarctica*, glutaraldehyde, tributyrine, and triglycerides standards were from Sigma-Aldrich. *Aspergillus niger* DSM823 and *Yarrowia lipolytica* DSM8218 strains were from DSMZ. Activity of biocatalysts was assayed by the hydrolysis of tributyrine with analysis of butyric acid by GC. Methanolysis of triglycerides was controlled by HPLC. The immobilization of microorganisms was controlled by SEM (see Fig.1, DSM8218 on haydite, bar 10 µm).



### **Conclusions**

The highest yield of biodiesel fuel (fatty acid methyl esters) was observed for the DSM823 mycelium immobilized on polyurethane foam in packed bed bioreactor. This suggests the whole-cell biocatalysis in continuous mode bioreactors is the most promising approach in biodiesel fuel production.

This work is supported by Ministry of Science and Education of Russian Federation (project 13.74.2014/K)

**FEMS-1834**

**Microbes and alternative energy sources**

## **THE USE OF MIXED HYDROLYTIC BACTERIA IN THE PRODUCTION OF SUPPLEMENTS ENHANCING BIOGAS PRODUCTION PROCESS**

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### **Background**

During the degradation of the lignocellulosic biomass by hydrolytic bacteria many organic compounds such as: volatile fatty acids, simple carbohydrates and many intermediate metabolites are released. These compounds are used by successive groups of microorganisms involved in the subsequent stages of anaerobic digestion process.

### **Objectives**

The main aim of this study was (i) degradation of lignocellulosic biomass (maize silage) by mixed hydrolytic bacteria and (ii) production of supplements dedicated for enhanced biogas production.

### **Methods**

(I) Preparation of the supplements: aerobic degradation of lignocellulosic biomass (3% d.m. of corn silage) by mixed hydrolytic bacteria for 72 hours at 30°C at pH 7. Centrifugation and extraction of the supernatant from the culture. (II) Analysis of influence of produced supplements on anaerobic digestion. Lab-scale anaerobic digestion process. Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), total solids (TS), volatile solids (VS).

### **Conclusions**

In the presented work it was showed that the mixed hydrolytic bacteria can be used for preparation of supplements dedicated for the methane fermentation process. The results demonstrate that supplements produced by hydrolysis mediated by mixed hydrolytic bacteria, can increase the efficiency of biogas production up to 20-30%. Such supplements can be used in both well-working biogas plants in order to increase the efficiency of the biogas production process.

**FEMS-1846**

**Microbes and alternative energy sources**

**ANAEROBIC CO-DIGESTION OF HARDLY BIODEGRADABLE ORGANIC MATERIALS WITH THE USE OF MAIZE SILAGE**

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**Background**

Many organic residues are often used as a substrate in anaerobic digestion, but the efficiency of the process is determined by the level of biodegradability of waste materials. To increase digester gas production from hard-degradable compounds various co-digestion process are applied. If co-substrates are used in anaerobic digestion system it improves the biogas yields due to positives synergisms established in the digestion medium and the supply of missing nutrients.

**Objectives**

The main aim of this study was to verify if maize silage, which is the most popular substrate used in production of biogas in agriculture plants, can be also a valuable co-substrate used for utilization of hardly biodegradable substrates such as sewage sludge waste (SSW) or residues from coal mines (RCM).

**Methods**

Laboratory anaerobic digestion process in various ratio corn silage and utilization of waste (3%, 2+1%, 1+2%, 0%). Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), total solids (TS), volatile solids (VS). Determination of total cell counts by fluorescence microscope, using the filter set WU for DAPI detection.

**Conclusions**

Obtained results showed that maize silage can be used as valuable co-substrates during utilization of various industrial waste materials. The addition of only 1% of the dry matter of maize silage to sewage sludge waste and residues from coal mine increased the biogas yield (even up to 300%).

**FEMS-1480**

**Microbes and alternative energy sources**

## **CHARACTERIZATION OF AN ANAEROBIC THERMOPHILIC GLYCEROL-DEGRADING ENRICHMENT CULTURE**

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### **Background**

The glycerol market was totally changed by the biodiesel industry, which resulted in the production of an excess of this compound as an industrial by-product. As a consequence, the price of glycerol dropped and a huge interest in alternatives for its valorisation emerged since then. In the field of Biotechnology research, glycerol is an attractive compound for the microbial production of chemical building blocks.

### **Objectives**

The aim of this work was to investigate thermophilic anaerobic communities capable of conversion of glycerol.

### **Methods**

Thermophilic sludge from a lab-scale anaerobic reactor fed with skim milk and sodium oleate (50:50% chemical oxygen demand) was incubated at 55°C in closed bottles containing bicarbonate-buffered medium supplemented with 10mM glycerol. Periodic successive transfers of the glycerol-converting enrichment culture, combined with serial dilutions were performed. After eight generations a highly enriched, low diversity (microscopic observations and 16s rRNA DGGE profiling) microbial culture was obtained.

### **Conclusions**

The enriched culture converted glycerol mainly to methane (6mM) and acetate (7mM) within 6 days of incubation. A yet unknown organic compound was also produced. Sequencing results obtained on the Illumina platform showed the bacterial predominance of an uncultured *Thermotoga* species (75 % of the retrieved sequences), an uncultured *Anaerobaculum* species (13 %) and a close relative to *Thermoanaerobacter pseudethanolicus* (5 %). Isolation of the new uncultured *Thermotoga* and *Anaerobaculum* species is ongoing and their role in glycerol degradation will be assessed.



**FEMS-2793**

**Microbes and alternative energy sources**

**SIMULTANEOUS AND SEPARATED SACCHARIFICATION OF BANANA PSEUDO-STEM WASTE (MUSA X PARADISIACA VAR. SAPIENTUM L.) AND FERMENTATION OF THE HYDROLYSATE USING MICROBE CONSORSIUM FOR BIOETHANOL PRODUCTION**

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**Background**

*Bioethanol fermentation can using the high cellulose substrat such as banana pseudo-stem that has 35,96% of cellulose content. The research about simultaneous and separated saccharification of banana pseudo-stem waste (Musa x paradisiaca var. sapientum L.) and fermentation of the hydrolysate for bioethanol production to get an effective enzyme dosage of hydrolysis , consortium of microbes, method of fermentation has been carried out.*

**Objectives**

The purpose of the research are to get the most effective concortium and method in bioethanol fermentation from banana pseudo-stem waste (*Musa x paradisiaca var. sapientum L.*) using *Simultaneous Saccharification and Fermentation* (SFS) and *Saparated Hydrolisis and Fermentation* (SHF) methods.

**Methods**

*The experimental method was used in fermentation process of banana pseudo-stem waste with Complete Randomize Design (CRD) consist of 2 factors and 3 replications. First factor were the combination of method and consorsium (K) and second factor were sampling time (T).*

**Conclusions**

*The result showed that the most effective dosage during dosage optimization of  $\alpha$ -amylase enzyme was dosage 1 with Dextrose Equivalent (DE) about 23,1; hemicellulase enzyme was dosage 2/3 with DE about 34,9; cellulase enzyme and glucoamylase enzym were dosage 2/3 with DE about 57,4. The result showed that concortium of *Pichia stipitis* and *Saccharomyces cerevisiae* and *Simultaneous Saccharification and Fermentation* (SFS) method were the best concortium and method in bioethanol fermentation of banana pseudo-stem waste that produce the highest ethanol concentration about 6,376% in 72 hours fermentation.*

## **FEMS-0917**

### **Microbes and alternative energy sources**

#### **ISOLATION OF THERMOPHILIC BACTERIA FROM VINASSE OF SUGAR CANE**

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#### **Background**

Vinasse is a liquid by-product of the ethanol production. Its high organic content (COD of 20-40g/L) means that its disposal into the environment is hazardous and has a considerable pollution potential. However, vinasse can be treated by fermentation process in bioreactors. In this case, thermophilic anaerobic digestion can be used to treat this wastewater from sugar cane industry.

#### **Objectives**

The aim of this work was to isolate thermophilic fermentative bacteria present in vinasse.

#### **Methods**

Thermophilic bacteria was isolated by anaerobic serial dilution technique on plates containing fermented vinasse with pH of 6,5 and at 50°C. Colonies were identified by MALDI-TOF mass spectrometry technique.

#### **Conclusions**

*Bacillus thermoamylovorans*, facultatively anaerobic and amylolytic bacterium was isolated from fermented vinasse. This strain ferments carbohydrate producing lactate, acetate, ethanol, and formate but not hydrogen (Combet-Blanc et al, 1995). The presence of this bacterium shows that vinasse is a source of microorganisms that can be used as inocula in anaerobic treatment of vinasse.

## **FEMS-1681**

### **Microbes and alternative energy sources**

#### **ENDO-BETA-1,4-MANNANASES FROM THERMOTOLERANT BACILLUS SP.**

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#### **Background**

Endo- $\beta$ -1,4-mannanases are important catalytic agent in several industries. The enzymes randomly cleave the  $\beta$ -1,4-linkage in mannan backbone and release short  $\beta$ -1,4-mannooligosaccharides and mannose. They are classified as glycoside hydrolases.

#### **Objectives**

The present study, the endo- $\beta$ -1,4 mannanase was isolated from thermotolerant bacteria and physicochemistry properties of purified enzyme were characterized.

#### **Methods**

Microorganisms (320 isolates) were screened on minimum medium containing locust bean gum at 45°C. The species of selected strain was identified by 16S rDNA sequence analysis. An endo- $\beta$ -1,4-mannanase was purified to homogeneity by using anion-exchange, hydrophobic, and size-exclusive column chromatographies. The molecular mass was analyzed by SDS-PAGE.

#### **Conclusions**

The 16S rDNA sequence revealed that the highest mannanase producing microorganism was *Bacillus* sp. The specific activity of purified enzyme was 14.5 U/mg. The apparent molecular mass was 38 kDa. The optimal pH and temperature for enzyme activity were pH 6.0 and 60°C, respectively. The enzyme was stable in a pH ranges from 5 to 9, after 16 h of incubation at 4°C and stable up to 60°C for 1 h, and more than 80 % of initial activity remained. This endo- $\beta$ -1,4-mannanase can be applied in several industries such as animal feed, pharmaceutical, paper and pulp, coffee, and bioethanol.

**FEMS-0646**

**Microbes and alternative energy sources**

## **ESCHERICHIA COLI [NI-FE]-HYDROGENASES ACTIVITY DURING GLYCEROL FERMENTATION UPON FORMATE SUPPLEMENTATION**

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### **Background**

*Escherichia coli* encodes four [Ni-Fe]-hydrogenases (Hyd).

### **Objectives**

H<sub>2</sub> producing Hyd activity was studied during glycerol fermentation at different pHs upon external formate supplemented (10 mM).

### **Methods**

H<sub>2</sub> evolution was measured by using pair of Pt and Ti-Si redox electrodes.

### **Conclusions**

At pH 7.5 and 6.5 wild type cells showed similar H<sub>2</sub> production rate ( $V_{H_2}$ ) when in the assays glycerol was added, whereas in formate supplemented assays it was decreased ~1.5 fold at pH 6.5.  $V_{H_2}$  was lowered ~2 fold in *selC* (coding for formate dehydrogenases) single mutant at pH 7.5. At both pHs when formate was added in the assays,  $V_{H_2}$  decreased in the strains where Hyd-3 or formate hydrogen lyase 1 related enzymes were disturbed. When glycerol was supplemented in the assays,  $V_{H_2}$  was significantly lowered in triple *hyaB hybC* (coding for large subunits of Hyd-1 and Hyd-2, respectively) *selC* or *hyaB hybC hycE* (coding for large subunit of Hyd-3) mutants at both pHs.

Taken together it might be suggested that Hyd-3 becomes mainly responsible for H<sub>2</sub> production during glycerol fermentation when external formate is added. Besides, in the glycerol supplemented assays, three hydrogenases can work in H<sub>2</sub> producing mode and only deletion of three of them decreases the production of H<sub>2</sub>. This effect might be due to disturbance of H<sub>2</sub> cycling. Therefore, responsible Hyd enzymes have been revealed under the conditions above.

All this is of significance in application of different carbon sources, especially mixed carbons, in H<sub>2</sub> production technology using bacteria

## **FEMS-3012**

### **Microbes and alternative energy sources**

#### **PURIFICATION OF FERULIC ACID ESTERASE FROM BACILLUS MEGATERIUM**

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#### **Background**

Ferulic acid esterase (FAE) are a subclass of carboxylic acid esterases (EC 3.1.1.73) that play role in complete enzymatic hydrolysis of hemicelluloses by hydrolyzing the ferulate ester groups in the cross-linking between hemicelluloses and between hemicellulose and lignin.

#### **Objectives**

To screen and purify ferulic acid esterase from thermotolerant bacteria

#### **Methods**

A total of 250 microorganisms collected from soil and rotten wood were isolated on minimum medium containing 1% rice straw at 45°C for strains that produced high FAE activity. The selected isolate was identified by 16S rDNA sequence analysis. Enzyme was purified by using anion-exchange, hydrophobic, and size-exclusive column chromatographies. The purified enzyme was characterized for its temperature and pH optima and stability.

#### **Conclusions**

The 16S rDNA analysis of selected isolate revealed that it belong to *Bacillus megaterium*. The optimum temperature and pH of purified FAE were 60°C and pH 7.0. The enzyme was stable in a wide pH ranges (pH 1–8), more than 80 % of initial activity remained after 16 h of incubation at 4 °C and stable up to 80°C for 1 h. The specific activity was 298.01 U/mg. This ferulic acid esterase have potential applications for several industries that high temperature and wide pH range are needed.

**FEMS-1266**

**Microbes and alternative energy sources**

**EFFECT OF PHOSPHOGYPSUM AND SEWAGE SLUDGE FERTILIZATION ON HEMP (CANNABIS SATIVA) YIELD AND MYCORRHIZA AND SOIL RESPIRATION, MICROBIAL BIOMASS AND DEHYDROGENASE ACTIVITY**

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**Background**

Phosphogypsum and sewage sludge are formed as a by-product during production of fertilizers from phosphate rock and industrial or municipal waste-water treatment, respectively.

**Objectives**

The aim of present study was to investigate the hemp yield and arbuscular mycorrhizal fungi (AMF) root colonization and spore formation in response to phosphogypsum and municipal sewage sludge fertilization. Additionally the effect of phosphogypsum and sewage sludge on soil respiration, microbial biomass and dehydrogenase activity were also examined.

**Methods**

The field spots with three hemp varieties Beniko, Białobrzeskie and Tygra were studied. An equivalent of 170 kg per ha pure nitrogen was used as fertilization in treatments with sewage sludge. The phosphogypsum at the level 100, 500 and 1000 kg per ha was applied.

AMF root colonization by microscopic and molecular techniques were studied. For determination of soil microbial biomass fumigation-extraction method (PN-ISO 14240-2) was used. Soil respiration by determination of CO<sub>2</sub> release by titration (PN-ISO 16072) and dehydrogenase activity by TTC method (PN-ISO 23753-1) were measured.

**Conclusions**

Application in agriculture seems to be one of the most reasonable possibility for utilization of phosphogypsum and sewage sludge, especially as fertilizer in energetic plant production. Additionally, phosphogypsum in combination with sewage sludge significantly increased soil biological activity.

Financial support was provided by EU Project at Warsaw Life Sciences University 'A program to improve the level of the didactic approach to the question of how to obtain raw plant materials for the purposes of energy production in the context of the Europe 2020 Strategy objectives'.



FEMS-2986

Microbiology of aquatic ecosystems

**PALLERONIA ABYSSALIS SP. NOV., ISOLATED FROM THE DEEP MEDITERRANEAN SEA AND THE EMENDED DESCRIPTION OF THE GENUS PALLERONIA AND OF THE SPECIES PALLERONIA MARISMINORIS**

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**Background**

Three strains designated 221-F1<sup>T</sup>, 221-F2 and 3030-F1 were isolated from the Matapan Vavilov Deep canyon, also known as Calypso Deep in the Eastern Mediterranean Sea, at a depth of 4,908 meters.

**Objectives**

To describe a new species based on 16S rRNA gene sequence analysis and phenotypic characteristics. These strains were found to be most closely related to *Palleronia marisminoris* and *Hwanghaeicola aestuarii*.

**Methods**

The description is based on a polyphasic approach with extensive phenotypic, quimiotaxonomic, genotypic and phylogenetic analysis.

**Conclusions**

The strains were observed to be red-pigmented and to form non-motile cocci or pleomorphic cells. The cells were found to stain Gram-negative, to be strictly aerobic, oxidase and catalase positive. Strains 221-F1<sup>T</sup>, 221-F2 and 3030-F1 were found to be mesophilic and to grow in medium containing up to 13 % NaCl. The major polar lipids of the three strains were identified as diphosphatidylglycerol, phosphatidylglycerol, phosphatidylcholine, an unidentified glycolipid and an unidentified aminolipid. Ubiquinone 10 (U-10) was found to be the major respiratory quinone. The DNA G+C content of strain 221-F1<sup>T</sup> was determined to be 64.7 mol %. The new isolates were unambiguously more closely related to the type strain of *Palleronia marisminoris* (95.3% of similarity) than to *Hwanghaeicola aestuarii* (94.7% of similarity). Based on phylogenetic, physiological and biochemical characteristics we describe a new species represented by strain 221-F1<sup>T</sup> (=CECT 8504<sup>T</sup> =LMG 27977<sup>T</sup>) for which we propose the name *Palleronia abyssalis* sp. nov. We also propose to emend the description of the genus *Palleronia* and the species *Palleronia marisminoris* to reflect new results obtained in this study.



**FEMS-1777**

**Microbiology of aquatic ecosystems**

## **ANAEROBIC SULFATE-REDUCING BACTERIA IN THE OXIC WATER COLUMN OF THE BLACK SEA**

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### **Background**

The Black Sea is a world's largest meromictic basin, where deep sulfide-rich waters do not mix with the upper oxic waters. Interestingly, sulfate-reducing bacteria (SRB) were detected there not only in the anoxic zone but also in the oxic water layer. A pure culture of a new SRB species, possessing different systems of antioxidative defense, was isolated for the first time from the Black Sea oxygenated water column.

### **Objectives**

We analyzed the phylogenetic composition of SRB community in the Black Sea oxic waters where representatives of *Desulfovibrio-Desulfomicrobium*, *Desulfococcus-Desulfonema-Desulfosarcina* and *Desulfotomaculum* subgroups were revealed. The pure culture of psychrophilic Gram-negative SRB was isolated from the subsurface oxic waters and described as *Desulfofrigus euxinos*. This new species possesses different systems of antioxidative defense including O<sub>2</sub> reduction and ROS detoxification.

### **Methods**

FISH, nested and qRT-PCR with primers to 16S rRNA and *dsrB* genes, DGGE and nucleotide sequencing were used to investigate the SRB community. DNA-DNA hybridization, fatty acids analysis and growth tests with different electron donors/acceptors were performed to characterize the SRB species. Oxygen consumption rates were measured with a Clark-type electrode. Key genes of antioxidative defense were identified by DOP-PCR, and inverse PCR was carried out for the sequence analysis of the entire genes.

### **Conclusions**

While some SRB are resistant to aerobic conditions because of multi-component antioxidative mechanisms, their presence in the sea oxygenated waters is likely to depend also on inhabiting anoxic microniches within suspended organic particles. The work was supported by RFBR projects nos. 10-04-00220-a and 12-04-91052-CNRS-a (PICS #6041).

**FEMS-1081**

**Microbiology of aquatic ecosystems**

## **SPATIO-TEMPORAL DYNAMICS OF BACTERIAL COMMUNITIES IN A STRATIFIED LAKE**

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### **Background**

Bacteria play an essential role in food webs and biogeochemical cycles in aquatic ecosystems. Investigating ecological relationships among bacterial taxa and between abiotic and biotic ecosystem components is critical to environmental control and functional traits of individual bacterial group. However, such information is scarce. Here we present the results of a study on the spatio-temporal dynamics of bacterial communities in a stratified lake.

### **Objectives**

The main objectives of the present study were to: (a) investigate the bacterial diversity and dynamics through time and space; (b) identify relations between specific bacterial groups and environmental parameters.

### **Methods**

We collected over a year monthly samples throughout the water column and from the sediment of a holomictic lake in the Netherlands. Subsequently, we sequenced the 16S rRNA genes of bacteria on an Illumina MiSeq platform to determine the diversity and relative abundance. Simultaneously different physical and chemical parameters were measured. Statistical analysis was performed to detect possible interactions among different populations and to determine relations between specific bacterial groups and environmental parameters.

### **Conclusions**

All sequences were affiliated to members of phyla commonly found in freshwater systems. Bacterial community changed dramatically over time and space in the water column, but clear seasonal successions could be observed. Contrastingly, the bacterial community in the sediment was quite stable, and mainly composed of *Proteobacteria* and *Bacteroidetes*. Statistical analysis showed *Actinobacteria* were positively correlated with temperature, while *Cyanobacteria* and *Planctomycetes* co-varied with nitrate. *Betaproteobacteria*, *Deltaproteobacteria*, *Gammaproteobacteria*,

*Epsilonproteobacteria* and *Firmicutes* were positively correlated with ammonium and negatively correlated with dissolved oxygen and pH.

**FEMS-2088**

**Microbiology of aquatic ecosystems**

**EXAMINATION OF THE RARE BIOSPHERE IN MICROBIAL MATS FROM SALAR DE HUASCO, AN ANDEAN HIGH ALTITUDE (3820 M) SALINE WETLAND**

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**Background**

The high-altitude wetlands of the Chilean Altiplano (Atacama Desert) are unique and remote aquatic ecosystems, and are considered part of the cold biosphere. These ecosystems characteristically contain organisms with a high level of endemism that can prosper under extreme environmental conditions. Furthermore, microbial diversity is typically high, and includes a large contribution of previously undescribed groups of Bacteria and Archaea. Microbial mats are common, but are typically small in area and depth relative to those described from other environments.

**Objectives**

To describe members of the rare biosphere from a micro-profile taken from Salar de Huasco microbial mat.

**Methods**

Bacterial diversity was described by pyrosequencing of 16S rRNA genes of each layer (5) of a microbial mat (<5 mm depth) as well as metagenomic analysis (454 Roche) of microbial mat samples. Oxygen microprofiles were performed *in situ* using a microprofiling system (Unisense). Microbial diversity was characterized by the presence of groups of low relative abundance (rare biosphere) and also low sequence identity with sequences of available databases. At the phyla level, rare bacteria from the microbial mats (1.6-0.05% of relative abundance) were affiliated with *Planctomycetes*, *Verrucomicrobia*, *Chlorobi*, *Acidobacteria*, SR1, *Armatimonadetes* (ex-OP10), OD1, BRC1, *Saccharibacteria* (ex-TM7), OP11, *Lentisphaerae*, *Spirochaetes*, *Synergistetes*, and others.

**Conclusions**

Different members of the rare biosphere were present across the microbial mat micro-profile. Particular rare groups could be associated with the specific conditions in each layer of the microbial mat, providing clues to their likely metabolic function.

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**FEMS-1963**

**Microbiology of aquatic ecosystems**

**MICROBIAL COMMUNITIES IN OPERATIONAL DRINKING WATER  
DISTRIBUTION SYSTEMS: IMPLICATIONS FOR DRINKING WATER QUALITY**

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**Background**

Microorganisms inhabiting drinking water distribution systems (DWDS), particularly those attached to pipes forming biofilms, can affect the safety and quality of the water. The presence of unfavourable microorganisms in DWDS is influenced by a number of factors ranging from physico-chemical properties of the source water to the characteristics of the pipe infrastructure itself. The difficulty of accessing the internal surface of pipes has limited the study of the microbial ecology of DWDS. Most previous research in this area has been based on laboratory models and/or assessing only few selected microorganisms under controlled conditions which do not represent real systems.

**Objectives**

To better understand the microbial ecology of DWDS, microbial communities were monitored in two chlorinated networks supplied with different source waters.

**Methods**

Biofilm sampling devices fitted with coupons designed to maintain boundary layer conditions at the pipe water interface were installed in the networks. Over a one year-period, water physico-chemical parameters were measured and the dynamics of plankton and biofilm communities were monitored using Illumina MySeq sequencing.

**Conclusions**

Clear differences in the composition of the microbial communities supplied with different source waters were detected. Microbial diversity in the groundwater supplied network was higher than previously thought in chlorinated systems. Source water and the hydraulic regime in the system were critical factors shifting microbial communities and influencing the quality of the water. This new understanding of the microbial ecology of DWDS is vital to improve control and management strategies to help safeguard drinking water quality and ultimately public health.

**FEMS-1362**

**Microbiology of aquatic ecosystems**

**CYTOTOXIC AND ANTIMICROBIAL ACTIVITIES OF SECONDARY METABOLITES PRODUCED BY MARINE ACTINOMYCETE STRAINS ISOLATED FROM SAUDI COASTAL HABITATS**

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**Background**

Cancer and infectious diseases are still the main two health problems facing the humankind. Actinomycetes from the coastal habitats of Saudi Arabia can represent a good source for drug discovery.

**Objectives**

The main objectives of the present study were screening the marine actinomycete strains isolated from the coastal habitats of Saudi Arabia for production of anticancer and antimicrobial compounds and identifying them to the species level by the polyphasic approach.

**Methods**

Eight samples were collected from the Arabian Gulf habitats of Saudi Arabia. The selective isolation of actinomycetes was carried out on three different recommended media. Twenty six strains were selected to study their diversity by examining their morphology and determining their cell wall diaminopimelic acid type and whole-cell sugar pattern. Sixteen representative strains were screened for their cytotoxic and antimicrobial activities and their taxonomic positions were confirmed by the phylogenetic analysis of the 16S rRNA gene sequence.

**Conclusions**

Based on dissimilarities in their appearance on two media, twenty six strains were selected and primarily assigned to 8 actinomycete genera; *Micromonospora*, *Streptomyces*, *Nocardiopsis*, *Amycolatopsis*, *Microtetraspora*, *Nonomuraea*, *Actinopolyspora* and *Saccharomonospora*. These results showed that there is good actinomycete genus diversity in the Saudi coastal habitats. Out of the selected sixteen strains, seven strains showed promising activity against the tested Gram-positive, Gram-negative bacteria and yeast strains. In addition, the actinomycete extracts showed potent anticancer activities against human T cell leukemia (Jurkat) and human laryngeal carcinoma (Hep-2) cell lines. The results are encouraging as novel strains have been isolated and identified and the active secondary metabolites are promising.



**FEMS-0909**

**Microbiology of aquatic ecosystems**

**MICROBIAL IRON CYCLING IN COASTAL MARINE SEDIMENTS –  
COMPETITION AND INTERRELATION OF FE(II)-OXIDIZERS AND FE(III)-  
REDUCERS**

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**Background**

Iron is an abundant redox-active element in many environments and iron(III)-reducing and iron(II)-oxidizing bacteria are largely responsible for microbial iron redox cycling. Three metabolic types of neutrophilic Fe(II)-oxidizing bacteria are known, i.e. microaerophilic, nitrate-reducing, and phototrophic Fe(II)-oxidizers. Based on the geochemical conditions in redox-stratified marine sediments, all three can potentially co-exist with overlapping niches.

**Objectives**

Consequently, there is potential for competition for their electron donor, Fe(II), that is formed by Fe(III)-reducing microorganisms which can interact with the iron-oxidizing microbial community. However, most studies of iron cycling have so far focused only on a single metabolic type of iron(II) oxidation coupled to iron(III) reduction.

**Methods**

In our study, we therefore determined the spatial distribution of the different metabolic types of iron-oxidizing and iron-reducing bacteria along the steep redox gradients within a coastal marine sediment from Aarhus Bay, Denmark, by MPN studies, isolations and qPCR. Furthermore, the iron(II)-oxidizing and iron(III)-reducing activity of the different metabolic types and their potential contribution to iron mineral formation within the sediment were quantified by microcosm studies.

**Conclusions**

From our results we conclude that microbial iron cycling occurs in these sediments and involves microaerophilic, phototrophic and nitrate-reducing Fe(II)-oxidizers as well as Fe(III)-reducers. The quantification of rates of iron oxidation and reduction under dynamic environmental conditions, including day-night cycles, input of nitrate or O<sub>2</sub>, reveals the competition and ecological network of iron-oxidizing and -reducing bacteria and is an essential step in order to evaluate the importance of microbial iron cycling in coastal marine sediments.



**FEMS-0825**

**Microbiology of aquatic ecosystems**

**DIVERSITY AND BIOSYNTHETIC POTENTIAL OF CULTURABLE YELLOW-PIGMENTED BACTERIA ASSOCIATED WITH THE SURFACE OF ANTARCTIC MACROALGAE**

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**Background**

Algal surfaces are an untapped source of microbial diversity and could represent a promising source of novel therapeutic agents. Very little is known about the diversity and bioactive potential of Gram-positive bacteria associated with the surface of Antarctic macroalgae. Furthermore, it remains unclear the natural distribution, diversity and ecological contributions of Antarctic pigmented heterotrophic bacteria. To our best knowledge, this study is the first research carried out on the diversity and biosynthetic potential of pigmented bacteria associated with Antarctic macroalgae.

**Objectives**

This study aims to explore the phylogenetic diversity and potential to produce secondary metabolites of Gram-positive, yellow-pigmented bacteria isolated from the surface of intertidal and subtidal Antarctic macroalgae.

**Methods**

Representative species of green, red and brown algae were collected from the intertidal and subtidal zone (5 and 30 m) of King George Island, Antarctica, in January 2014. Surface-associated bacteria were investigated by cultivation-based methods and 16S rRNA gene sequencing. Yellow-pigmented isolates were screened for the presence of genes encoding polyketide synthases (PKS) by PCR amplification with degenerate primers.

**Conclusions**

The phylogenetic analysis showed that the yellow-pigmented epibionts belonged to the genera *Arthrobacter*, *Citricoccus*, *Kocuria*, *Labeledella*, *Microbacterium*, *Salinibacterium* and *Staphylococcus*. PKS sequences were detected in *Kocuria* and *Staphylococcus* isolates. It highlights that Antarctic macroalgae are a unique source of microbial diversity for natural product research.

**Acknowledgments**

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**FEMS-2160**

**Microbiology of aquatic ecosystems**

**STUDYING THE GREAT POTENTIAL OF CULTIVABLE BACTERIAL  
COMMUNITIES ASSOCIATED WITH THE BROWN ALGA ASCOPHYLLUM  
NODOSUM**

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**Background**

Bacteria associated with algae differ markedly from those living freely in seawater and represent great potential for the production of diverse bioactive compounds as they interact in multiple complex ways with their host.

**Objectives**

This work aims to study cultivable bacteria associated with the brown alga *Ascophyllum nodosum*, showing polysaccharolytic activities.

**Methods**

To isolate cultivable microorganisms, algal thalli of *Ascophyllum nodosum* were swabbed with sterile cotton tips and marine agar plates were inoculated. Three-hundred isolated bacteria were screened for agarase, kappa- or iota-carrageenase activities on specific marine media. Thirty-two bacteria with polysaccharolytic activities were isolated and a part of their 16S rDNA (8F-1492R) were amplified and sequenced. Twenty-seven were classified as *Flavobacteriia* and five as *Gammaproteobacteria*. Putative new strains and species of *Zobellia*, *Maribacter*, *Cellulophaga*, *Shewanella*, *Glaciecola*, *Pseudoalteromonas* and *Colwellia* were identified by phylogenetic analysis. Genomics libraries with their DNA were constructed in *Escherichia coli* and *Bacillus subtilis* and are currently screened for diverse enzymatic activities (agarases, iota-and kappa-carrageenases, cellulases, beta-glucosidases, sulfatases and amylases).

**Conclusions**

In an era where high throughput sequencing is mostly used to study bacterial communities, cultivation methods are underestimated. Here, we revealed that only ten percent of the cultivable bacteria on this brown alga could degrade algal polysaccharides, which lead to asking us; who and what are the 90 other percents doing there? Furthermore, by this cultivation method we could also identify putative new bacterial strains/species, which are screened for polysaccharidases. Novel glycoside hydrolases from unknown marine bacteria represent great biotechnological potential as they should have original industrial properties.



**FEMS-0922**

**Microbiology of aquatic ecosystems**

**COMBINING MORPHOLOGICAL, BIOCHEMICAL AND PHYSIOLOGICAL ASPECTS TO COMPARE TWO STRAINS OF MICROCYSTIS AERUGINOSA (CYANOBACTERIA) DIFFERING IN THE PRODUCTION OF MICROCYSTIN**

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**Background**

*Microcystis aeruginosa* is a bloom forming cyanobacteria impacting freshwater environments worldwide. Blooms are formed by a mix of strains, some able to produce a hepatotoxic peptide called microcystin, others not.

**Objectives**

Our objective was to compare two *M. aeruginosa* strains, a microcystin producing and a non producing one, isolated from reservoirs in São Paulo, Brazil.

**Methods**

The strains were cultured (23±2 °C, 40-50 μmol photons m<sup>-2</sup>.s<sup>-1</sup>, 14-10h light–dark cycle, ASM-1 medium), cells collected at exponential growth phase and morphological and physiological aspects were investigated. Proteomic shotgun analysis was performed using iTRAQ and 2DLC-MS/MS.

**Conclusions**

The strains were similar in chlorophyll-a and phycocyanin content, the non toxic strain had higher carotenoid content. The toxic strain was richer in antioxidants. It formed floating colonies with numerous cells embedded in mucilage (diameter 150-800μm). In contrast, the non toxic strain exhibited small colonies (100μm) with few cells dispersed in thick mucilage, not floating. Electronic microscopy revealed thylakoids and polyphosphate granules equally present in both strains, aerotopes more abundant in the toxic one, cyanophycin granules and carboxysomes more evident in the non toxic strain. Sixty nine differentially expressed proteins were found. Both strains expressed proteins related to photosynthesis, energy metabolism and translation. Gas vesicle and stress response proteins were more abundant in the toxic strain while transport and protein folding functions were more expressed in the non toxic one. These results are discussed in relation to the other physiological aspects investigated. These differences probably represent distinct ecological strategies adopted by each strain.

**FEMS-1699**

**Microbiology of aquatic ecosystems**

**BIODEGRADATION OF MICROCYSTIN-LR BY BACTERIA FROM THE SEDIMENT OF JACAREPAGUA LAGOON (RIO DE JANEIRO, BRAZIL)**

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**Background**

The release of effluents into aquatic systems leads to an increase in nutrients, a process known as eutrophication. This process promotes large proliferations of cyanobacteria (blooms) that can be harmful to the environment and to humans due to the production of cyanotoxins. The cyclic peptide microcystin (MC) is the prevalent and most studied cyanotoxin in blooms, with more than 80 variants. It has been reported consistently since 1996 in Jacarepagua Lagoon (RJ). MC is rarely found dissolved in the water column, but adsorbs to particles in suspension which settle at the bottom sediment or undergo biodegradation by microorganisms.

**Objectives**

The objective of this study was to evaluate the potential for biodegradation of MC-LR by bacteria found in the lagoon sediment, testing both recently isolated strains and microbial assemblages.

**Methods**

MC-LR was incubated with isolated strains (15) or microbial assemblages for 1 and 7 days, and quantified by LCMS.

**Conclusions**

After 7 days only one strain showed degradation of MC. The microbial assemblage extracted directly from the sediment was very efficient in degradation, decreasing (by ~50%) the added MC. Part of the observed decrease in MC amount was recognized as adsorption to sediment particles. The 15 isolated strains were identified as *Ralstonia* or *Bacillus* by 16SrDNA sequencing. This indicated that the culture medium led to selection of microorganisms, probably eliminating those able to degrade MC. On the other hand, MC degradation by microbial assemblages reflects the metabolic diversity of this sample and points to a possible cooperative activity to complete the process of biodegradation.

**FEMS-0633**

**Microbiology of aquatic ecosystems**

**MOLECULAR CHARACTERIZATION OF PSEUDOMONAS AERUGINOSA  
POPULATION IN AQUATIC ENVIRONMENTS OF GREECE**

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**Background**

Recent isolation of *Ps. aeruginosa* strains from Greek aquatic environments and subsequent PFGE analysis demonstrated that these strains have a unique genotype. Environmental isolates may also have a unique phylogenetic position. Literature has revealed that the behavior of *Ps. aeruginosa* during typing is affected by its habitat and by stress factors such as antibiotics, biofilm formation e.t.c.

**Objectives**

Considering the advantages of MLST, as a typing method that focuses strictly on 7 conserved housekeeping genes, it was chosen for typing and discrimination of *Ps. aeruginosa* strains isolated from water samples from all over Greece. Additional information regarding the resistant phenotypes circulating in these environments is cited.

**Methods**

The activity of 14 antibiotics was tested against all isolates by the disk diffusion method, where additional phenotypical tests were performed according to published protocols. MLST was performed as described by Curran et al, 2004 with some alterations concerning the annealing temperatures of the seven housekeeping genes. The control strains *Pseudomonas aeruginosa* ATCC 27853, clinical control from HPA/NEQAS (External Quality Control) and PAO1 were used as standards of reference during the MLST and for reproducibility experiments.

**Conclusions**

The majority of the isolates exhibited the intrinsic antimicrobial resistance, while a significant proportion presented additional resistant mechanisms. The amended protocol of MLST seemed to produce allelic profiles for all the isolates so far tested. Thus this typing method is going to be used in a larger sampling program to obtain more data and to conclude in a reliable phylogenetic analysis for the first time in aquatic environments of Greece.





**FEMS-1864**

**Microbiology of aquatic ecosystems**

**PHOTOCHEMICAL AND MICROBIAL ALTERATIONS OF DOM SPECTROSCOPIC PROPERTIES IN THE ESTUARINE SYSTEM RIA DE AVEIRO**

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**Background**

Photochemical transformations of chromophoric dissolved organic matter (CDOM) influence microbial communities in aquatic systems.

**Objectives**

This work aimed to evaluate this influence in the estuary Ria de Aveiro. In order to achieve that, two sites representative of the marine (MZ) and brackish water (BZ) zones of the estuary were regularly surveyed.

**Methods**

Optical parameters of CDOM indicative of aromaticity and molecular weight were used to establish CDOM sources, and microbial abundance and activity was characterized. Additionally, microcosm experiments were performed in order to simulate photochemical reactions of CDOM and to evaluate microbial responses to changes in CDOM composition.

**Conclusions**

The CDOM of the two zones showed different spectral characteristics, with significantly higher values of the specific ultra-violet absorbance at 254 nm and of the absorption coefficient at 350 nm and lower SR ratio at BZ than at the MZ, reflecting the different amounts and prevailing sources of organic matter, as well as distinct riverine and oceanic influences. At the MZ, the abundance of bacteria and the aminopeptidase activity correlated with absorbance, suggesting a microbial contribution to the HMW CDOM pool. The irradiation of DOM resulted in loss of color and increase of its bioavailability. However, the extent of photoinduced transformations and microbial responses was dependent on the initial characteristics of CDOM. In Ria de Aveiro both photochemical and microbial processes yielded optical changes in CDOM and the overall results of these combined processes determine the fate of CDOM in the estuary and have an influence on local productivity and in adjacent coastal areas.

**FEMS-1519**

**Microbiology of aquatic ecosystems**

**IN SITU DETECTION AND LOCALIZATION OF THE PROBIOTIC *PEDIOCOCCUS* SP. AB1 IN THE GUT OF ABALONE *HALIOTIS GIGANTEA***

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**Background**

We previously reported that host-derived *Pediococcus* sp. Ab1 can colonize the gut of abalone *Haliotis gigantea* and that the addition of Ab1 to commercial feed can alter gut microflora and increase alginate lyase and VSCFAs in the gut. From those viewpoints, addition of Ab1 can provide valuable effects in abalone aquaculture as well as in other aquaculture animals.

**Objectives**

In this study, to revealing the dynamics of probiotic *Pediococcus* Ab1, we tried to establish an optimum FISH protocol, and tried to *in situ* detection of this bacterium.

**Methods**

TAMRA-labeled Rpt probe was constructed and samples were fixed by 4 % paraformaldehyde and hybridized at 40 °C for 3 h. In the case of described condition, we could discriminate clearly Ab1 from other control bacterial strains (*Enterococcus*, *Lactobacillus*). Fluorescence signals of Rpt probe-positive bacteria were easily distinguished from any detectable background autofluorescence.

**Conclusions**

Rpt probe-positive bacteria were detected in both probiotics supplemented animal and non-probiotics supplemented animal. Rpt probe-positive bacteria formed microbial colonies only in the probiotics supplemented animal. The mean numbers of Rpt probe-positive bacteria in the gut-attached samples of probiotic supplemented animal was  $1.94 \times 10^7$  cells/g-gut, and those of non-probiotic supplemented animal were  $6.85 \times 10^5$  cells/g-gut, respectively. From the thin section FISH analysis, Rpt positive cells were observed from gut samples of probiotics supplemented animal. Interestingly, Rpt positive cells were also observed from gills samples of them. From these results indicated that probiotic *Pediococcus* sp. ab1 is colonized and make a micro-colony in their host gut.

**FEMS-0747**

**Microbiology of aquatic ecosystems**

**REDUCTION OF BIOFILM FORMATION IN COOLING TOWER WATER SYSTEMS USING NANO-HYDROPHOBIC COATING**

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**Background**

The proposed study is to reduce biofilm formation using nano-hydrophobic coating on cooling tower fill materials. Consequently, enhancing cooling performance, lengthen the material life, reducing clogging and biofilm associated pathogen bacteria.

**Objectives**

Primary aim is to reduce the slimy biofilm formation on fill material, which will lead to better cooling of water, longer material life and less clogging of the fills. It is widely known that cooling towers' maintenance is heavily neglected. This end ups in shorter material life, expensive maintenance charges, inefficient cooling, excessive energy consumption and public health risk due to Legionella bacteria dissemination. While novel anti-biofilm approaches are still in nascent phases of development, our efforts are devoted for sustainable management of cooling towers and public health safety, where cooling towers are often associated with deadly Legionnaires' disease.

**Methods**

Test surfaces were placed into biofilm reactor along with the untreated control coupons up to 6-months period for biofilm maturation. Natural bacterial communities were monitored to analyze the impact to mimic the real-life conditions. Surfaces were monthly analyzed *in situ* for their microbial load using epifluorescence microscopy.

**Conclusions**

Wettability is known to play a key role in biofilm formation on surfaces, because characteristics of surface properties affect the bacterial adhesion. Results showed that surface-conditioning with nano-silica significantly reduce (up to 90%) biofilm formation. Easy coating process is a facile and low cost method to prepare hydrophobic surface without any kinds of expensive compounds or methods.

**FEMS-1840**

**Microbiology of aquatic ecosystems**

**THE REGULATION OF DIMETHYLSULFONIOPROPIONATE METABOLISM IN RUEGERIA POMEROYI DSS-3**

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**Background**

Dimethylsulfoniopropionate (DMSP) plays a key role in the global sulfur cycle and marine microbial sulfur and carbon metabolism. The marine roseobacter *Ruegeria pomeroyi* DSS-3 is capable of metabolizing DMSP by either the cleavage or demethylation pathways. The cleavage pathway forms the volatile gas dimethylsulfide (DMS), the largest natural source of sulfur to the atmosphere. The demethylation pathway produces methanethiol (MeSH), which is readily assimilated or oxidized.

**Objectives**

A model for the regulation of DMSP metabolism has been developed for *R. pomeroyi* where the availability and turnover of methyl-tetrahydrofolate (THF) is a major regulatory point.

**Methods**

A combination of RNA-Seq, enzyme assays and growth experiments were performed to test predictions of the model.

**Conclusions**

According to RNA-Seq, the genes involved in THF biosynthesis and methyl-THF metabolism were significantly up-regulated during growth on DMSP as compared to acetate. Similarly, the specific activities of the methylene-THF reductase and formate dehydrogenase were two-fold higher in cell-free extracts of DMSP-grown cells. During growth on DMSP, treatment of cultures with low concentrations of the dihydrofolate reductase inhibitor trimethoprim increased production of DMS without inhibiting growth. This result was consistent with a model where high levels of methyl-THF metabolism allow DmdA to function at its maximal rate, leading to sufficient MMPA accumulation to overcome the DMSP-based inhibition of RPO\_DmdB2, one of the two isozymes for the second step in the pathway. Based on the current

knowledge of DMSP metabolism, the regulation of the two pathways in *R. pomeroyi* appears to be complex and multifaceted.

**FEMS-0570**

**Plant/microbes interactions**

## **EFFECT OF BOTANICAL EXTRACTS & ESSENTIAL OILS IN FEED ON LAYING HENS PERFORMANCE & SALMONELLA, E.COLI & TOTAL BACTERIAL COUNT IN FECES**

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### **Background**

*Ph.d Research idea was to explore plant extracts effects*

### **Objectives**

The aim of the present study was to investigate the effect of Botanical Extracts and Essential oils on performance of laying hens

### **Methods**

One hundred and twelve, 40 weeks old, white Novagin laying hens were randomly assigned to seven groups equally (n = 16). Each treatment (group) was replicated four times of four birds per replicate. Experimental diets were prepared by adding A: positive control, antibiotic lincomycin 4.4%, 120mg/kg of feed, antioxidant seldox (BHA, BHT, ethoxyquine and citric acid) 120mg/kg of feed, acetic acid 99.5% pure 0.15ml/kg of feed), B: negative control (N.C) , no antibiotic, antioxidant and acetic acid, C: N.C + black tea poly phenolic extract 1ml/kg of feed, D: N.C + black cumin seed poly phenolic extract 1ml/kg of feed, E: N.C + fenugreek seed poly phenolic extract 1ml/kg of feed, F: N.C + black cumin seed oil 1ml/kg of feed, and G: N.C + fenugreek seed oil 1ml/kg of feed.

### **Conclusions**

Weekly egg production percentage of positive control is significantly higher than negative control ( $P < 0.05$ ) but plant extracts and oils has no significant difference between each other ( $P > 0.05$ ). On the other hand Total bacterial count significantly reduced with increasing age in all treatments except negative control but there is no significant difference between treatments when compared for presence of salmonella and E. coli ( $P > 0.05$ ) in feces.

**FEMS-2391**

**Plant/microbes interactions**

**EFFECT OF ROOT EXUDATES AND DIFFERENT CARBON SOURCES ON BACTERIAL GROWTH OF BACILLUS AMYLOLIQUEFACIENS FZB42 AND BACILLUS SUBTILIS BBG131 AND THEIR LIPOPEPTIDE PRODUCTION**

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**Background**

Rhizosphere is the region that is closed to the roots of plant. Plant root exudates consist of a complex mixture of organic acids, sugars and amino acids, which are used as substrates for the growth of bacteria colonizing this rhizosphere.

**Objectives**

we highlighted on the effect of root exudates and some of carbon sources which found in the root exudates, on the bacterial growth and lipopeptides production

**Methods**

Ten of carbon sources (glucose, fructose, sucrose, maltose, xylose, glutamic acid, citric acid, succinic acid, oxalic acid, fumaric acid), and root exudates were used to study bacterial growth and lipopeptides production

**Conclusions**

Different growth kinetics were observed depending of the substrates. For both strains, growth on oxalic acid is very slow and glucose is the best substrate. As a conclusion, the different behavior of the two strains to consume the root exudates and the different carbon sources, which reflex to the growth and lipopeptides production, may be causing the different ability to colonize the rhizosphere.

**FEMS-3013**

**Plant/microbes interactions**

## **ABUNDANCE AND DIVERSITY OF FUNGAL ENDOPHYTES FROM THE GOTJAWAL FORESTS ON JEJU ISLAND IN KOREA**

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### **Background**

Endophytes are microorganisms that inhabit plant tissues by colonizing internal plant tissues without causing apparent harm to host. Particularly, some fungal endophytes have beneficial roles on plant growth and plant tolerances to environmental stresses such as abiotic and biotic stresses. Accordingly, identifying their abundance and diversity from unique forest ecosystem is an interested subject to discovery the beneficial fungal endophytes.

### **Objectives**

The objective of this study is to construct endophytic fungal resources for discovering their beneficial functions, by collecting diverse fungal endophytes from the Gotjawal forest (Jeju, Korea), a unique forest ecosystem, and identifying their abundance and diversity.

### **Methods**

Various plant tissues were collected from Gotjawal area on Jeju Island in Korea. They were sterilized and then, subjected to the endophytic fungal isolation. Subsequently, they were further pure-cultured and then subjected to the long-term storage in liquid nitrogen and the DNA isolation for the molecular identification, mediated by various DNA barcode markers.

### **Conclusions**

Forty seven plant samples were collected from five Gotjawal forests on Jeju Island and composed of 34 species. Through the molecular identification, 196 fungal isolates were obtained from the plant tissues and classified as 3 classes, 11 orders, 17 families and 29 genera. Especially, fifty fungal MOTUs (molecular operational taxonomic units) were identified and classified further into four genera such as *Colletitrichum*, *Xylaria*, *Pestalotiopsis*, *Fusarium*. The fungal collection, obtained from Gotjawal forests, was deposited with classification information in National



Institute of Biological Resources as stocks and can become a useful resource to discovery the beneficial functions of fungal endophytes.

**FEMS-2401**

**Plant/microbes interactions**

**DOES INTERACTION OF THE INVASIVE CORDGRASS SPARTINA DENSIFLORA WITH PLANT GROWTH-PROMOTING RHIZOBACTERIA EXPLAIN ITS TOLERANCE TO PHYSICOCHEMICAL PROPERTIES OF MARSHES SOILS?**

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**Background**

Microbiota in the niches of the rhizosphere zones can affect plant growth and responses to environmental stress conditions via mutualistic interactions with host plants.

**Objectives**

A glasshouse experiment was designed to investigate the role of a bacterial consortium (*Pseudomonas composti* SDT3, *Aeromonas aquariorum* SDT 13 and *Bacillus* sp. SDT14) isolated from the rhizosphere of *Spartina densiflora* in its growth and physiological tolerance to the physicochemical properties of marshes soils.

**Methods**

Plants of *S. densiflora* were randomly assigned to two soil types with different physicochemical characteristics with and without inoculum for 50 days. Plant responses were examined using growth analysis, combined with measurements of gas exchange, efficiency of PSII biochemistry, total content of photosynthetic pigments and leaf water content. In addition the accumulation of nutrients in roots and leaves were determined.

**Conclusions**

The inoculation improved growth of *S. densiflora* through a beneficial effect on its photochemical apparatus due to its impact on chlorophyll concentration. This growth enhancement happened under both soil conditions and was mainly reflected in a greater length and diameter of roots. Modifications of pigment concentrations were linked to an increase in leaf magnesium content. Also, inoculation favoured LWC through the decline in gs and increment in root-to-shoot ratio. Moreover, this consortium was able to stimulate ions uptake in roots and leaves. Plant growth-promoting rhizobacteria of *S. densiflora* appears to play a significant role in its growth response and tolerance to the physicochemical properties of soils, through diverse protective effects on the photosynthetic apparatus, WUE and mineral nutrient balance.

**FEMS-2084**

**Plant/microbes interactions**

**BACTERIAL CONSORTIA FORMULATED WITH ENDOPHYTIC AND RHIZOSPHERIC BACTERIAL STRAINS, ISOLATED FROM AVOCADO PLANTS, INCREASE SALT STRESS TOLERANCE OF WHEAT SEEDLINGS.**

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**Background**

The plants growing under salinity stress conditions increase ethylene production, which induces root elongation inhibition. The bacterial enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACCD) cleave ethylene precursor, the 1-aminocyclopropane-1-carboxylate, thus mitigating ethylene effects. Some strains also produce phytohormone indol acetic acid (IAA), which increases cell elongation.

**Objectives**

To determine the effects of inoculation with consortia formulated with IAA- and ACCD-producing endophytic (EB) and rhizospheric bacteria (RB) on salinity stress mitigation of wheat plants.

To compare the effects of EB and RB inoculation, as well as, different levels of bacterial IAA and ACCD production on salinity stress mitigation of wheat plants.

**Methods**

Twelve bacterial strains were isolated from avocado plants, and four consortia were formulated: 1) EB with higher ACCD and IAA production; 2) EB with lower ACCD and IAA production and 3) RB with higher ACCD and IAA production; 4) RB with lower ACCD and IAA production. Wheat seeds were inoculated with the bacterial consortia, and then seeds were grown under salt stress conditions. Length, dry weight and superoxide dismutase (SOD) activity of wheat shoot and roots were determined.

**Conclusions**

At lower levels of bacterial IAA and ACCD production, the EB were more efficient than RB consortia mitigating salt stress effects. Between RB consortia, only those with higher production are able to promote the growth of stressed plants. Both EB and RB consortia with higher production are able to increase SOD activity. Finally,

bacterial strains isolated from avocado plants mitigate plant stress and therefore have the potential to be used as commercial inoculum of avocado plants.

**FEMS-2688**

**Plant/microbes interactions**

## **CALLOSE DEPOSITION AND ACCUMULATION OF STARCH IN CITRUS AND PONCIRUS LEAVES INFECTED WITH “CANDIDATUS LIBERIBACTER ASIATICUS”**

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### **Background**

Huanglongbing (HLB) is a destructive disease of citrus caused by phloem-limited bacteria, *Candidatus Liberibacter* spp. Although no known HLB-resistant citrus species or varieties have been identified, several studies report the group of *Poncirus trifoliata* and some of its hybrids, as more tolerant to the disease. One of the early manifestations of HLB is excessive starch accumulation in leaf chloroplasts.

### **Objectives**

We hypothesize that callose deposition in the phloem may intervene photoassimilate exportation, causing the starch to over-accumulate. Thus, we examined citrus leaf phloem by microscopy to characterize plant responses to infection.

### **Methods**

We have studied 3 genotypes (*C. sinensis*, *C. sunki* and *P. trifoliata*) and 7 hybrids obtained from crosses between *C. sunki* and *P. trifoliata*. All genotypes (3 replicates) were graft inoculated with budwood from HLB-infected plants and infection was confirmed by qPCR (TaqMan®) 8 months after plants inoculation.

### **Conclusions**

We observed callose deposition in leaf petioles and accumulation of starch in leaves in all genotypes and hybrids, when compared with mock-inoculated controls. However, accumulation of starch and callose deposition were more abundant in *C. sinensis*, *C. sunki* and three of the hybrids. *P. trifoliata* and four of the hybrids showed the lowest starch accumulation and lower deposition of callose, when compared with others genotypes. Our results indicate that *Liberibacter* infection is accompanied by callose deposition in different level among the genotypes and the deposition of callose is higher in susceptible genotypes suggesting that the phloem plugging by callose inhibits phloem transport, contributing to the development of HLB symptoms.

**FEMS-1816**

**Plant/microbes interactions**

**PYROSEQUENCING ANALYSIS OF SOIL FUNGAL COMMUNITIES OF  
DIFFERENT TRANSFORMATION SYSTEMS IN THE JAMBI PROVINCE  
(SUMATRA, INDONESIA)**

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**Background**

In many tropical areas rainforests are cleared in order to transform them into rubber and oil palm plantations, although massive transformations are a major threat for biodiversity. Most of the tree species in rainforests are associated with fungi that form mutualistic interactions with roots and function as key link for nutrient exchange between the above- and belowground compartment. Therefore, in addition to the loss in tree species, transformation of primary forests into intensely managed plantations may lead as well to changes in the fungal community structure of soils or even to a loss of species.

**Objectives**

The goal of this study is to identify potential differences in fungal community structures in soils of tropical lowland rainforests and plantations sites. Furthermore soil-borne plant stress will be evaluated by analyzing arbuscular mycorrhizal (AM) spore density and morphology.

**Methods**

The study is implemented in one of the largest regions of tropical lowland rainforest in Southeast Asia, the Jambi Province (Sumatra, Indonesia). Two landscapes, National Park Bukit Duabelas and Harapan Rainforest, were investigated. Soils of different transformation systems (lowland rainforest, jungle rubber (extensive), intensive rubber and oil palm plantations) were investigated using the 454 Pyrosequencing approach. Fungal soil communities were characterized using internal transcribed spacer regions ITS1 and ITS2.

**Conclusions**

A strong increase of AM spore density in plantations compared to rainforest sites was detected supporting that monoculture tree crops may suffer from soil-born stress.

Currently we are investigating if transformation of tree species rich tropical rainforests to species poor plantations correlates with a loss of fungal diversity in soil.

**FEMS-1225**

**Plant/microbes interactions**

## **ROLE AND ACTIVITY OF MIRNAS IN THE INTERACTION OF TOMATO ROOTS WITH ENDOPHYTIC POCHONIA CHLAMYDOSPORIA**

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### **Background**

MicroRNAs (miRNAs) are endogenous small-RNAs transcribed from non-coding DNA, matching a target messenger RNA to repress translation or induce cleavage. They act in almost every biological plant activity, e.g. development, abiotic stress tolerance, signal transduction, and in defense from pathogens or parasites.

### **Objectives**

To elucidate miRNAs role in plant-endophyte interactions, we constructed libraries from roots of *Solanum lycopersicum* endophytically colonized (Pmi) or not (Pm) by the hyphomycete *Pochonia chlamydosporia*. This fungus shows endophytic behaviour with growth promotion or nematode biocontrol effects. No data are available on tomato miRNAs role and targets in the endophytic interaction.

### **Methods**

Illumina™ NGS of small-RNAs yielded  $9 \cdot 10^6$  (Pmi) and  $12 \cdot 10^6$  (Pm) reads per library. CLC Genomics Workbench was used for trimming, counting, annotation and data analysis.

### **Conclusions**

Non-redundant, unique small-RNAs (869178 in Pmi, 958026 in Pm), were produced. MiRNAs expression was affected by endophytism. Analyses of tomato miRNAs (miRBase, rel.21), revealed miR156 and miR168 (conserved across higher plants), as most abundant in roots. Four further miRNAs (miR169a, miR169c, miR9473 and miR9476), out of 75 known in tomato, were expressed only in Pmi, with seven further (miR169d, miR1917, miR169e, miR394, miR167a, miR5300 and miR9475) over-expressed and 27 down-regulated (fold change range: 1.2–4.8). 37 remaining miRNAs were equally expressed in both conditions. A Pmi comparative analysis showed that 1732 out of 5055 Pmi down-regulated genes were miRNA targets,



involved in structural protein, metabolism, transcription factor, growth and development, stress-related, signaling pathways, storage and other processes.

**FEMS-0916**

**Plant/microbes interactions**

**CHARACTERIZATION OF THREE ENSIFER MELILOTI BACTERIOPHAGES FROM SOILS SUBJECTED TO ABIOTIC STRESS.**

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**Background**

The presence of bacteriophages in soils was demonstrated as early as 1935. The presence of rhizobiophages in soils suggests that through selection or elimination of certain types of rhizobia, the rhizobiophages influence the evolution of bacterial populations.

**Objectives**

In this work, we isolated 3 *Ensifer meliloti* bacteriophages (EMph1, EMph2 and EMph3) from the rhizosphere soil of *Medicago marina* collected in two areas of the South of Spain. This study is part of a project in which the diversity of rhizobia strains that nodulate *M. marina* was studied in the Odiel river marshes (Huelva) and in the dunes of San Fernando beach (Cádiz), soils subjected to pH and salt stress.

**Methods**

Bacteriophages were isolated from rhizosphere soil samples by the enrichment technique of Barnett (1972). Phages were purified by three successive isolation of single plaques. The basic DNA manipulations and molecular techniques used have been described elsewhere. To study their host range, phages were assayed on 22 different bacteria, 13 of them were strains isolated from *M. marina* nodules, along with additional bacteria, including strains of *E. meliloti*, *E. medicae*, *E. fredii*, *R. tropici*, *R. etli*, *R. leguminosarum* bv. *viciae*, *R.l.* bv. *trifolii* and *Agrobacterium tumefaciens*.

**Conclusions**

The 3 phages were resistant to restriction with many enzymes tested. EMph1 and EMph3 displayed a wide host range within the whole bacteria tested. On the contrary, phage EMph2 exhibited a narrow host range which was capable of killing only 5 out of 22 bacteria tested.

Barnet (1972). J. Gen. Virol. 15:1-15.



**FEMS-1623**

**Plant/microbes interactions**

**THE NOVEL ADVANCES IN UNDERSTANDING OF PECTOBACTERIUM  
ATROSEPTICUM SCRI1043 STRATEGIES DURING PLANT-MICROBE  
INTERACTIONS.**

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**Background**

Many phytopathogenic bacteria colonize heterogeneous interior of their host consisting of various tissues that form diverse microniches for microorganisms. However, the dynamics of bacterial population structure in such heterogeneous and changing system as plant organism is poorly understood.

**Objectives**

In our investigations we described the dissociation of *Pectobacterium atrosepticum* SCRI1043 (*Pba*) populations and the formation of various subpopulations in tobacco plants.

**Methods**

Microbiological methods; RT PCR; light, transmission electron microscopy; immunocytochemistry; chromatography.

The proliferative potential and bacteria distribution within different plant tissues were analyzed from inoculation time till long-term preservation of microorganisms in dead plant debris. We have revealed new structures totally occluding xylem vessels of the host plant and termed them "bacterial emboli". These structures are composed of tightly packed bacterial cells having a predominant spatial orientation and a peculiar way of formation. Using immunocytochemistry and biochemical methods it was shown that high molecular weight products of pectic compounds degradation and *Pba* exopolysaccharides play an important role in the bacterial emboli formation. Such structures were formed in the plant vessels both in the pronounced and inapparent disease symptoms after *Pba* infection. Bacterial emboli are likely to form conditions for bacterial downward migration through the xylem vessels resulting in rhizosphere colonization. After plant death bacteria were transformed to viable but non-culturable state.

**Conclusions**

Our investigation demonstrates that pectobacteria realize different strategies in colonization of various plant compartments and are able to pass through the life cycle symptomatically and asymptotically.

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**FEMS-3060**

**Plant/microbes interactions**

**THE NOVEL ADVANCES IN UNDERSTANDING OF PECTOBACTERIUM ATROSEPTICUM SCRI1043 STRATEGIES DURING PLANT-MICROBE INTERACTIONS.**

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**Background**

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**Objectives**

In our investigations we described the dissociation of *Pectobacterium atrosepticum* SCRI1043 (*Pba*) populations and the formation of various subpopulations in tobacco plants.

**Methods**

Microbiological methods; RT PCR; light, transmission electron microscopy; immunocytochemistry; chromatography.

The proliferative potential and bacteria distribution within the different plant tissues were analyzed from inoculation time till long-term preservation of microorganisms in dead plant debris. We have revealed new structures totally occluding xylem vessels of the host plant and termed them "bacterial emboli". These structures are composed of tightly packed bacterial cells having a predominant spatial orientation and a peculiar way of formation. Using immunocytochemistry and biochemical methods it was shown that high molecular weight products of pectic compounds degradation and the *Pba* exopolysaccharides play an important role in the bacterial emboli formation. Such structures were formed in the plant vessels both in the pronounced and inapparent disease symptoms after *Pba* infection. Bacterial emboli are likely to form conditions for bacterial downward migration through the xylem vessels resulting in rhizosphere colonization. After plant death bacteria were transformed to viable but non-culturable state.

**Conclusions**

Our investigation demonstrates that *pectobacteria* realize different strategies in colonization of various plant compartments and are able to pass through the life cycle

symptomatically and asymptotically.

**FEMS-0986**

**Plant/microbes interactions**

## **COMPOSING PLANT-MICROBIAL ASSOCIATION RESISTANT TO SOIL SALINIZATION**

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### **Background**

Potash mining by Belaruskali concern poses a grave environmental threat in terms of soil pollution with sodium, potassium and calcium chlorides. Salt-induced stress affects growth and productivity of cultivars. A promising method for salinized soil remediation is field introduction of legume crops, like alfalfa (*Medicago sativa*) upgrading soil fertility due to atmospheric nitrogen fixation of selected rhizobial strains and PGRP bacteria. This work was aimed at isolation of beneficial microorganisms enhancing alfalfa resistance to elevated salt concentrations in soil.

### **Objectives**

Halophilic phosphate-solubilizing and nodulating bacteria.

### **Methods**

Recovery and selection of phosphate-solubilizing bacteria was conducted on TY medium containing chlorides of sodium, potassium, calcium in concentration range 3-15 %. Taxonomic affiliation of isolates was based on physiological-biochemical characterization using VITEK2 identification system.

### **Conclusions**

Four halophilic bacterial cultures able to grow on TY medium comprising 15% NaCl were isolated from specimens of solid saliferous wastes sampled at Starobin potash deposit. The examined isolates were capable to solubilize phosphates and exert favorable influence on seed germination, growth and development of alfalfa. Treatment of alfalfa seeds by strain FM-3 under saline conditions increased germination rate by 50% as compared to control. Nitrogen-fixing strain *Ensifer meliloti* Mst 3-2 isolated from alfalfa nodules displayed good growth on TY medium containing 2% NaCl. Investigation allowed to refer three phosphate-solubilizing isolates to genus *Bacillus*. The isolated strains of nitrogen-fixing and phosphate-solubilizing bacteria are especially attractive as components of plant-microbial association resistant to soil salinization.



FEMS-1380

Plant/microbes interactions

## CULTIVATING BIOFUEL CROPS ON MARGINAL LAND: PLANT-ASSOCIATED BACTERIA OF LIGNIN-REDUCED (GMO) ARABIDOPSIS THALIANA AND THEIR CAPACITY TO FACILITATE GROWTH.

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### Background

Producing second generation biofuels out of lignocellulosic biomass can contribute to reduce the food-fuel competition on fertile agricultural soils. However, the **recalcitrance of lignin polymers** is a limitation during processing the biomass to pure cellulose fibers. The efficiency of this process can be increased by using **genetically modified crops** with lower lignin-contents. Though, the genetic modification can have effects on the phenolic compounds inside the xylem which can influence the endophytic bacterial population.

To even further reduce the food-fuel competition, biomass can be cultivated on **marginal land** (containing e.g. cadmium (Cd)). An additional advantage can be the **reduction of contaminants** in these soils by making use of phytoremediation, a process that can be optimised by inoculating bacteria with plant growth promoting capacity (e.g. siderophores, IAA, organic acid and ACC deaminase production).

### Objectives

We want to identify the effects of the genetic modification and/or Cd-exposure on the endophytic bacterial population and determine the endophytic growth promoting capacity.

### Methods

The endophytes of different tissues of *Arabidopsis thaliana* wildtype and reduced lignin mutants, grown in a hydroponic system with and without 3µM CdSO<sub>4</sub>, were isolated and characterised using 16S rDNA amplification/sequencing. Their *in vitro* plant growth promoting capacity was tested using colorimetric tests.

### Conclusions

Differences in bacterial populations due to the genetic modification and Cd-exposure were observed in stems and seeds but they seemed to be less pronounced in leaves. The root endophytic populations were more or less identical. However, among all tissues differences in growth promoting capacity were found.

**FEMS-1328**

**Plant/microbes interactions**

## **DIVINYL ETHER SYNTHASE BRANCH OF LIPOXYGENASE CASCADE IN PLANT DEFENSE AGAINST PATHOGENS**

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### **Background**

Plants lack an immune system in the sense that it exists in animals. However, they are able to recognize potential pathogens. Lipoxygenase signaling pathway plays an important role in regulation of plant growth and development, cell signaling and defense. Lipoxygenase initiates synthesis of group of bioactive compounds collectively called oxylipins (fatty acid hydroperoxides, hydroxy-, oxo-, or keto-fatty acids, divinyl ethers, volatile aldehydes, or jasmonates). Usually oxylipins are not preliminary formed but synthesized de novo in response to mechanical damage, infection and other stress factors. The main role in the biosynthesis of plant oxylipins belongs to the CYP74 enzymes that are non-classical cytochromes P450: allene oxide synthases (AOS), hydroperoxide lyases (HPL), divinyl ether synthases (DES). A number of AOSs and HPLs have been cloned and characterized from various plant species. DESs are less studied. However, divinyl ethers were found in a large number of organisms.

### **Objectives**

We studied interaction between *Linum usitatissimum*, *Ranunculus acris* and *Selaginella moellendorffii* plants with pathogen bacteria *Pectobacterium atrosepticum* SCRI1043.

### **Methods**

Methods of microbiology, molecular biology, biochemistry, mass spectrometry, NMR and UV spectroscopy.

### **Conclusions**

We cloned and characterized DESs from *L.usitatissimum* (LuDES), *R.acris* (RaDES) and *S.moellendorffii* (SmDES1 and SmDES2). LuDES, RaDES and SmDES2 convert

substrates into divinyl ethers (omega5Z)-etherolenic and (omega5Z)-etheroleic acids, respectively. The main reaction product of SmDES1 is (11Z)-etherolenic acid. (Omega5Z)-etherolenic acid possesses bactericide properties, whereas other divinyl ethers – bacteriostatic properties. Expression of DESs genes after infection is 50 times higher than in control. Thus, DES branch of lipoxygenase cascade involved in plant defense against pathogens.

**FEMS-1287**

**Plant/microbes interactions**

## **BIOFILM FORMATION OF *RALSTONIA SOLANACEARUM* IS REQUIRED FOR ITS VIRULENCE**

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### **Background**

Cells of a bacterial pathogen *Ralstonia solanacearum* strain OE1-1 (OE1-1) form biofilms on surfaces of tomato cells adjacent to intercellular spaces after invading tomato plants, and cause bacterial wilt. OE1-1 cells in intercellular fluids but not xylem fluids from tomato plants form mushroom-type biofilms. The production of major extracellular polysaccharide, EPS I, positively regulated by a multi-transcription regulator PhcA is involved in the biofilm formation by OE1-1 cells.

### **Objectives**

We analyzed involvement of biofilm formation by OE1-1 cells in its virulence.

### **Methods**

Among PhcA-positively regulated genes, we created *raIA* encoding furanone synthetase-deleted mutant. Biofilm formation of OE1-1 and the mutant incubated in intercellular fluids from tomato plants were observed under the scanning electron microscope. EPS I production and expression of EPS I production-related genes, *xpsR* and *epsB*, were analyzed. Furthermore, virulence of the strains on tomato plants was analyzed.

### **Conclusions**

The mushroom-type biofilm formation of the *raIA*-deleted mutant significantly reduced, compared to those of OE1-1. Furthermore, expression of *xpsR* and *epsB*, and EPS I production of the mutant significantly reduced. Inoculation with the mutant into tomato plants using root dipping resulted in a significant reduction of bacterial growth in intercellular spaces and loss of its virulence. On the contrary, tomato plants inoculated with the mutant through petiole wilted. The transformation with native *raIA* resulted in recovery of not only EPS I production and biofilm formation but also virulence. These results suggest that biofilm formation of which regulation is positively influenced by *raIA* may be involved in OE1-1 virulence.

**FEMS-0639**

**Plant/microbes interactions**

## **EFFECTS OF RHIZOBACTERIAL ACC DEAMINASE ACTIVITY ON THE GROWTH OF SOYBEAN UNDER SALINE CONDITION**

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### **Background**

1-aminocyclopropane-1-carboxylate (ACC) deaminase is an enzyme that degrades the precursor of plant hormone ethylene. Plant growth-promoting bacteria that ACC-deaminase facilitate plant growth and development under stress conditions by decreasing plant ethylene levels.

### **Objectives**

This study was conducted to analyse the changes in root architecture and root hair length of soybean induced by three rhizobacteria *Pseudomonas putida* TSAU1, *Pseudomonas aureantiaca* TSAU22, *Pseudomonas* sp.. NUU8 strains containing ACC-deaminase.

### **Methods**

ACC-deaminase activity was determined by monitoring the amount of ammonia generated due to hydrolysis of ACC by the rhizobacterial isolates containing ACC-deaminase. Four salinity levels (5.0, 7.5, 10.0 and 12.5 dSm<sup>-1</sup>) were maintained in the gnotobiotic system using NaCl salt.

### **Conclusions**

Results showed that the salinity adversely affected on the root length of soybean decreasing by 79% the highest NaCl concentration (12.0 dSm<sup>-1</sup>). The bacterial strains could utilize ACC as N source indicating the presence of ACC-deaminase that play a role in reducing ethylene levels in plants. The inoculation of seeds with the *P.putida* TSAU1, *P. aureantiaca* TSAU22, *Pseudomonas* sp. NUU8, increased the root and shoot length of soybean at 5.0, 7.5 and 10.0 dSm<sup>-1</sup> up to 58% compared to uninoculated plants exposed to salt stress. In summary, based on the results of our work we recommend utilization of rhizobacteria having capacity to produce ACC-deaminase to alleviate salt stress of soybean grown in the saline soils.

**FEMS-0696**

**Plant/microbes interactions**

## **CO-INOCULATION OF BRADYRHIZOBIUM WITH PSEUDOMONAS STRAINS TO IMPROVE GROWTH AND YIELD OF SOYBEAN UNDER SALINE SOIL CONDITION**

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### **Background**

Salinity stress is one of the most serious factors limiting the productivity of agriculture, especially the negative effects on yield and nitrogen fixation of Leguminous plants. However, there are several reports on the positive impacts of co-inoculation with *Pseudomonas* and *Rhizobium* spp., but less attention has been paid how co-inoculation of root-colonising and PGPR bacteria will affect *Rhizobium-Legume* interactions under salinity stress.

### **Objectives**

The objectives of this research were to observe if the co-inoculation of soybean with *Bradyrhizobium japonicum* NU1 and *Pseudomonas* sp. NUU8 can enhance salt tolerance, nodulation, plant growth, pod yield and grain yield of soybean under saline soil conditions.

### **Methods**

The effects of bacterial inoculation on growth, nodulation, pod yield and grain yield of soybean plant grown under saline soil conditions were studied in field experiments. Plants were grown in saline soil of Sherobod district, Surkhandarya province, Uzbekistan.

### **Conclusions**

The results showed that co-inoculation of with *B. Japonicum* NU1 and *Pseudomonas* sp. NUU8 gave more benefits in nodulation, plant growth, pod yield and grain yield of soybean compared to plants inoculated with *B. japonicum* NU1 alone. Under field condition, co-inoculation of *B. japonicum* NU1 and *Pseudomonas* sp. NUU8 strains significantly improved shoot dry weight by 38% and root dry weight by 58% of soybean compared with the uninoculated control. The synergistic use of *B. japonicum* NU1 and *Pseudomonas* sp. NUU8 also improved the nodulation, plant growth, pod yield and grain yield under salt-stress. The results suggested that these strains could be used to formulate a biofertilizer for sustainable production of soybean under salt stressed field conditions.

**FEMS-1516**

**Plant/microbes interactions**

## **INDOLE ACETIC ACID BIOSYNTHESIS AND NITRIC OXIDE METABOLISM CROSSTALK IN AZOSPIRILLUM BRASILENSE SM**

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### **Background**

Plant growth promoting rhizobacteria, *Azospirillum brasilense* SM releases phytohormone-Indole-3-acetic acid (IAA) and other plant growth regulators into the rhizosphere which enhances plant development. A crosstalk between IAA and gasotransmitter, Nitric oxide (NO) is speculated which may further benefit the plants.

### **Objectives**

To identify NO production by strain SM and unravel potential rhizospheric crosstalk between IAA and NO.

### **Methods**

The study involved creating mutant strains which overexpress essential NO metabolism genes, NO Fluorescence assay, IAA quantification by HPLC, determining plant-bacterial association by SEM, and Real time PCR for gene expression.

### **Conclusions**

Production of NO and presence of NO metabolism genes i.e. nitrous oxide reductase (*nosZ*), nitrous oxide reductase regulator (*nosR*) and nitric oxide reductase (*norB*) were identified by fluorescence assay and PCR sequencing. Improved PGP response of their overexpressing mutant strains was mediated by increased NO and IAA levels. Surface colonization of strain SM on sorghum roots was established by electron microscopy and improved plant development was observed with the mutants. Quantitative IAA estimation suggested that *nosR* and *norBC* influences regulation of IAA biosynthesis in *A. brasilense* SM. The NO quencher, inhibitor and donor reduced or blocked IAA biosynthesis in wild type and mutants, emphasizing a common regulatory role of these molecules in IAA biosynthesis. Expression studies by qPCR showed positive influence of Tryptophan and Arginine on NO genes. IAA biosynthesis gene, indole-3-pyruvate decarboxylase (*ipdC*) was influenced by Tryptophan but not significantly by Arginine. These results impress some shared signalling mechanism or potential crosstalk involving IAA and NO in strain SM.



**FEMS-1726**

**Plant/microbes interactions**

## **IMPORTANCE OF METHANE FOR SPHAGNUM-ASSOCIATED MICROBIAL NITROGEN FIXATION**

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### **Background**

Pristine *Sphagnum*-dominated bogs are N-limited and depend on N<sub>2</sub> fixation by diazotrophs or atmospheric N-deposition for their N supply. *Sphagnum* mosses harbor a very diverse microbiome including numerous methanotrophs that contribute to the carbon supply of the mosses. It has been hypothesized that methane might also stimulate *Sphagnum*-associated N<sub>2</sub> fixation during early stages of peat development, thereby increasing *Sphagnum* growth and N content, although other studies did not see this effect.

### **Objectives**

The objectives are to elucidate whether methane indeed does stimulate N<sub>2</sub> fixation in *Sphagnum* mosses from ombro-, oligo- and mesotrophic field sites and to test whether this is affected by oxygen level.

### **Methods**

N<sub>2</sub> fixation and CH<sub>4</sub> oxidation activity were studied by incubating mosses with <sup>15</sup>N<sub>2</sub>, <sup>15</sup>N<sub>2</sub> + <sup>13</sup>CH<sub>4</sub> or no additions. Furthermore, different oxygen regimes (aerobic, microaerobic (either N<sub>2</sub>-He atmosphere or submerged) were applied. DNA and RNA are to be extracted and 16S rRNA and nitrogenase (nifH) genes quantified and analyzed by qPCR and high-throughput sequencing of amplicons.

### **Conclusions**

The isotope tracer-studies showed that only in oligotrophic sites the diazotrophic communities associated with *Sphagnum* reacted positively to methane addition and microaerobic conditions. For mosses from ombrotrophic and mesotrophic sites, diazotrophic activity was neither affected by methane addition, nor by reduced oxygen concentration. Combined, these results indicate that methane dependent nitrogen fixation may only be important under certain environmental conditions in *Sphagnum*-dominated peatlands. Conditions proven to be important for

methanotrophy (pH, water level) and diazotrophic community composition are likely to affect diazotrophy as well, and will be subject of future studies.

**FEMS-1951**

**Plant/microbes interactions**

**INTERACTION BETWEEN MICROALGAE AND BACTERIA - ANALYSIS OF COMMON MECHANISMS TO COMMUNICATE**

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**Background**

Algae live in close association with microorganisms. However, our knowledge on the physiology and metabolism of complex microbial communities associated with algae is very limited.

**Objectives**

A main goal of our research is to understand the underlying molecular mechanism of species divergence and adaptive processes in microalgae and bacterial systems. According to the fundamental questions "Who's there?" and "What are they doing?", bacterial communities associated with plants and their functions should be analyzed in more detail. We have analyzed the bacterial populations of different microalga and we speculate that these bacteria have evolved a common mechanism to communicate and manipulate their hosts.

**Methods**

The diversity and population dynamic of the bacteria were examined using scanning electron microscope, 16S rRNA gene analysis, MS and metagenome analyses. Moreover, the current work is focused on transcriptome analysis.

**Conclusions**

A comprehensive understanding of microalgae and bacteria interactions requires knowledge of the associated gene expression changes in both the microalgae and the bacteria. So, we will analyze the role of common signaling pathways and signals from eukaryotic and prokaryotic sites. The results will help us to understand overall principles of microbial adaptation and signal exchange in more detail.

**FEMS-1442**

**Plant/microbes interactions**

## **TRANSCRIPTOMIC ANALYSIS IN RHIZOSPHERIC BURKHOLDERIALES INTERACTING WITH ARABIDOPSIS EXUDATES**

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### **Background**

Bacteria are able to colonize a wide spectrum of habitats including the rhizosphere of plants. In such association bacteria may utilize plant root exudates components as carbon and energy source, but also other processes may take place, like degradation of toxics, competition with pathogenic bacteria, phytohormones synthesis, nitrogen fixation, quorum sensing control, and several others. Despite the relevance for agronomical applications, the molecular mechanisms underlying microbe-plant root exudates interactions are poorly understood.

### **Objectives**

To improve understanding of plant-microbe interactions through transcriptomica and metabolomics profiling of selected bacteria exposed to plant root exudates when plants have been grown under different nutritional and stress conditions.

### **Methods**

Cell cultures of two different Burkholderiales species able to associate with Arabidopsis (one plant growth promoting rhizobacterium –PGPR- and the other one a non-PGPR) were exposed to plant root exudates obtained from plants grown on control, N-limitation, and saline or chemical stress conditions, and gene expression profiling: Real Time PCR analysis for specific genes, and complete transcriptomic profile analysis by High-Seq Illumina platform, were obtained. Metabolomic profiling of plant root exudates was done by HPLC-MS.

### **Conclusions**

Plant root exudates exhibit different composition profile according to the growth conditions of the plant. This may explain differential gene expression profiles observed with the PGPR and the non-PGPR species.

**FEMS-2537**

**Plant/microbes interactions**

**ENDOPHYTIC BACTERIA THE INTROVERT ENGINEERS OF RHIZOSPHERE: A CASE STUDY EMPLOYING THEIR USE FOR REJUVENATION OF APPLE TREES IN SIX DECADE OLD APPLE ORCHARDS**

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**Background**

Apple cultivation a prominent industry in Himachal Pradesh, India occupies an area of 1,01,485 hectares and production reaching 2,11,295 metric tonnes. Today, the six decade old apple orchards are being replaced by new plantations. However, their survival is a major challenge which results in indiscriminate use of chemical inputs. This poses potential threat to environment/human health and therefore managing plant rhizosphere is critical.

**Objectives**

Work presented in here, highlights novelty of *Bacillus* sp. strain CKA1 (apple roots endophyte) in being used as rhizosphere engineer.

**Methods**

Microbial formulation was evaluated under field conditions for its effect on plant establishment; early growth; yield and rejuvenation of diseased apple trees. Whole genome sequencing of the bacterium helped in deciphering molecular mechanisms underlying the multifunctional plant growth promoting potential. Application of microbial formulation resulted in: increased plant biomass (75.40-80.11%); increased Nitrogen (2.42%), Phosphorus (0.58%) and Potassium (1.33%); increased fruit yields (35 to 45%); rejuvenation of diseased apple trees infected with *Dematophora necatrix* in farmer's field over a three year trial. Comparative genomics paved the way for understanding molecular mechanisms involved in the strains ability to function as multifunctional plant growth promoter.

**Conclusions**

Apple orchard rejuvenation projects are being ambitiously launched by State agencies for restoring six decade old planted apple orchards. Integrated nutrient management systems comprising biological systems especially, managing rhizosphere using endophytes such as strain CKA1 opens up new avenues not only for improving crop yield but also in sustaining soil health.

**FEMS-2368**

**Plant/microbes interactions**

## **POTENTIAL FIBRINOLYTIC ACTIVITY OF AN ENDOPHYTIC XYLARIA CURTA SPECIES FROM WESTERN GHATS OF INDIA**

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### **Background**

Endophytic fungi are prominent producers of putative phytochemicals and their analogues which can be exploited as therapeutic interventions/or pharmaceutical agents.

### **Objectives**

The current investigation deals with isolation, purification and characterization of fibrinolytic enzymes produced by endophytic fungal isolates derived from different medicinal plants of Western Ghats of India.

### **Methods**

In preliminary screening assays, 17% of the endophytic fungal isolates expressed fibrinolytic activity whereas 26% of the isolates exhibited proteolytic activity. Maximum fibrinolytic and proteolytic activity was shown by endophytic fungal isolate #37 CRSTBRT which exhibited a halo formation of 113.04 mm<sup>2</sup> on fibrin clot assay. The fibrinolytic enzyme (VM 22) from fungus was purified to electrophoretic homogeneity with the methods including ammonium sulphate precipitation, anion exchange and gel filtration chromatography. The molecular weight of the purified enzyme was estimated to be 35 KDa by SDS-PAGE, fibrin zymography and gel filtration chromatography. The enzyme was stable between 5.5 – 8.5 pH and below 50°C. The optimal pH for the enzymatic activity was 7.8 at 35°C. The purified enzyme also exhibited an 18 fold increase in the enzyme activity. *In vitro* studies also revealed that the enzyme could effectively catalyses the blood clot lysis. Further the endophytic fungal isolate #37 CRSTBRT was identified as a *Xylaria curta* species based on morphological and molecular taxonomic tools.

### **Conclusions**

The fibrinolytic enzyme VM 22 directly lyse the fibrin clot and not by plasminogen activators indicating that this enzyme could be useful in thrombolytic therapy.

**FEMS-3098**

**Plant/microbes interactions**

**EFFECT OF GENE SILENCING OF THIOREDOXIN H DURING THE COMPATIBLE INTERACTION OF CAPSICUM ANNUUM EUPHORBIA MOSAIC VIRUS-YUCATAN PENINSULA**

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**Background**

There are evidence of the participation of salicylic acid (SA) pathway in the plant/virus interaction. Thioredoxins (Trxs) participate as a regulator in this mechanism, and in plants, the *h*-type play a fundamental role in tolerance of oxidative stress and defense responses against pathogens.

**Objectives**

The objective of this work was to analyze the effect of silenced *CaTRXh1* in plants of *Capsicum annuum* during the compatible interaction with the begomovirus *Euphorbia mosaic virus-Yucatán Peninsula* (EuMV-YP).

**Methods**

CaTrxh1 was silenced in plants of *Capsicum annuum* using a VIGs vector based on the TRV. 15 days post-silencing, plants were infected with the EuMV-YP. A time course experiments was done. Plant samples were collected at different times post-inoculation. Gene expression of thioredoxin h, NPR1, and PR10, and EuMV-YP replication were evaluated on the time course by Real Time PCR.

**Conclusions**

*NPR1* and *PR10* genes increase their expression at 14 and 7 days post inoculation (DPI), respectively. In the silenced and infected plants, the expression of *NPR1* decreases in approximately 41% at 28 DPI, while *PR10* gene maintains the same expression at 7 DPI. At 4 hour post inoculation (HPI) it was observed an increase in the SA content in the plant infected with the EuMV-YP and at 1 HPI in the pTRV2:CaTRX/EuMV-YP, compared with the control plants. Also, the viral DNA accumulation was higher in the plants silenced and infected with the begomovirus. These results suggest the participation of *CaTRXh1* on plants of pepper in the mechanism of defense during the compatible interaction to EuMV-YP.



**FEMS-1288**

**Plant/microbes interactions**

## **INVOLVEMENT OF BIOFILM FORMATION OF *RALSTONIA SOLANACEARUM* IN ITS COLONIZATION IN INTERCELLULAR SPACES**

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### **Background**

*Ralstonia solanacearum* is a soilborne bacterial pathogen and is the causal agent of bacterial wilt of more than 200 plant species, limiting the crop production around the world. The colonization of *R. solanacearum* strain OE1-1 (OE1-1) in intercellular spaces after invasion through wounds is required for its virulence.

### **Objectives**

We analyzed colonization mechanism of OE1-1 in intercellular spaces.

### **Methods**

The behavior of GFP-labeled OE1-1 cells in intercellular spaces of tomato plants was observed under the fluorescence microscope. OE1-1 cells in intercellular spaces were then observed under the scanning electron microscope (SEM). OE1-1 cells in intercellular fluids and xylem fluids from tomato plants were observed under the SEM. Furthermore, biofilm formation of the major extracellular polysaccharide, EPS I, productivity-deficient mutants was also analyzed.

### **Conclusions**

The observation under the fluorescence microscope showed aggregation of fluorescence from GFP-labeled OE1-1 cells in intercellular spaces. The SEM observation showed attachment of OE1-1 cells on surfaces of tomato cells adjacent to intercellular spaces and biofilm-like structures by OE1-1 cells surrounded by an extracellular matrix. Interestingly, the SEM observation showed that OE1-1 cells incubated in intercellular fluids from tomato plants formed mushroom-type biofilms. On the contrary, incubation in xylem fluids led to a reduction of biofilm formation by OE1-1 cells. Furthermore, EPS I productivity-deficient mutants incubated in intercellular fluids significantly reduced their biofilm formation. Together, colonization of OE1-1 cells in intercellular spaces of tomato plants may consist of their attachment and biofilm formation on surfaces of tomato cells adjacent to intercellular spaces, in which EPS I productivity is involved.

## FEMS-2015

### Plant/microbes interactions

#### LEAF SPRAYING WITH RHIZOBACTERIA FOR BIOLOGICAL CONTROL OF XANTHOMONAS AXONOPODIS PV. PHASEOLI

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#### Background

The bean common blight (BCB) is the most important bacterial disease in the tropics. The chemical control of the BCB is neither efficient nor economic. Therefore, is important to develop new approaches to control CBB. Previous studies using seed microbiolization showed that DFs strains and combinations were effective in control leaf, root and vascular diseases (CORRÊA et al., 2014) and changed enzymatic activities (SILVA et al., 2009).

#### Objectives

So, here these rhizobacteria were evaluated by spraying them in leaves inoculated with *Xanthomonas axonopodis* pv. *phaseoli*.

#### Methods

The treatments were *Bacillus* (DFs093, DFs348 and DFs769), *Pseudomonas* (DFs513 and DFs831) and their combinations (DFs93+DFs769+DFs831 and DFs348+DFs769+DFs831) or water (test) sprayed 48 or 24 h before, 24 or 48 h after inoculation of the pathogen. The experiment was conducted in a greenhouse, in a design completely randomized, with six repetitions. The BCB severity was assessed after 96 hours each two days using a diagrammatic scale (0 to 6).

#### Conclusions

The test plants were 100% symptomatic after 120 hours and the final severity was between 5.35 and 5.48. Only the spraying 48 h before the pathogen inoculation result in a significant difference between the bacterial treatments. The treatments DFs513, DFs769, and the combination DFs348+DFs769+DFs831 showed a smaller area under disease progress curve (AUDPC) (20.6, 26.8 and 22.8% respectively). This result suggests the occurrence of induced resistance, since it requires previous spraying, as occurs when these treatments were used to treat seeds.

CORRÊA et al., 2014. Biological Control, 72: 71-75.

SILVA et al., 2009. Summa Phytopathologica, 35: 98-104.

**FEMS-2879**

**Plant/microbes interactions**

## **THE RHIZOSPHERE MICROBIOME OF SEAGRASSES AND THEIR ROLE IN SULFUR PROCESSES**

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### **Background**

Seagrass meadows grow in coastal, biogeochemically active sediments. These productive ecosystems are distributed from tropical to temperate areas and are dominated by seagrasses, a group of marine angiosperms that is often threatened by the presence of hydrogen sulfide, a phytotoxic gas produced by belowground bacterial communities. The seagrass rhizosphere is still poorly described, however the occurrence of die-off events caused by high levels of sulfide stresses the need to understand these plants and bacterial communities present in their rhizosphere – rhizobiome.

### **Objectives**

The aim of this research was to obtain insight into the seagrass rhizobiome and to answer the following questions: a) Is the rhizobiome plant-specific?; b) Does the rhizobiome co-evolve with its host?; and c) Who are the core species of the seagrass rhizobiome?

### **Methods**

Rhizosphere samples of *Zostera marina*, *Z. noltii* and *Cymodocea nodosa*, bulk sediments and seawater were collected from Portugal, and the former two rhizospheres were additionally collected from France. Next Generation Sequencing of 16S rDNA amplicons was performed using MiSeq Illumina platform.

### **Conclusions**

The rhizobiome of seagrasses differs significantly from their surrounding environment. Although they don't vary at a local scale, the rhizobiomes were significantly different between plants from different geographical locations. The core rhizobiome of seagrasses is mainly composed of OTUs involved in the sulfur cycle (sulfate reduction and sulfur oxidation), and known for their ability to fix nitrogen.

Moreover, our results point to a niche differentiation of sulfur bacteria, in which sulfide oxidation is performed by different taxa.

**FEMS-2913**

**Plant/microbes interactions**

## **EFFECT OF GFP-LABELED PAENIBACILLUS POLYMYXA ON GROWTH OF AGRICULTURAL CROPS**

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### **Background**

*Paenibacillus polymyxa* strain P2b-2R isolated from internal stem tissue of a naturally regenerating pine seedling fixes nitrogen in association with lodgepole pine seedlings and promotes their growth. It has been shown to colonize lodgepole pine seedling tissues endophytically using a green fluorescent protein (GFP)-labelled derivative of P2b-2R.

### **Objectives**

We wanted to see if the GFP labelled derivative of P2b-2R would fix N and promote growth of corn and canola in ways similar to the wild type strain.

### **Methods**

We inoculated corn and canola seeds with wild type P2b-2R or the GFP labelled derivative of P2b-2R and seedlings were grown for 40 days in a N-limited soil mix. Seedlings were harvested 20, 30 and 40 days after inoculation and evaluated for biological nitrogen fixation and growth promotion.

### **Conclusions**

Seedlings inoculated with the GFP labelled P2b-2R strain derived small amounts of N from the atmosphere (upto 17%) but grew significantly larger than seedlings inoculated with wild type P2b-2R. Seedlings growth was promoted by inoculation with GFP labeled P2b-2R with an increase of upto 40% in height and 70% in biomass as compared to wild type P2b-2R. Thus, we concluded that GFP modification of strain P2b-2R resulted in a significant enhancement of its growth promotion efficacy of corn and canola.

**FEMS-2911**

**Plant/microbes interactions**

**CAN AN ENDOPHYTE ISOLATED FROM LODGEPOLE PINE TREES RESIDE INSIDE AGRICULTURAL CROPS AND FIX N?**

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**Background**

Several bacterial strains of *Paenibacillus* that possessed N-fixing ability were isolated from extracts of surface-sterilized lodgepole pine seedling and tree tissues. One strain, *Paenibacillus Polymyxa* P2b-2R, was found to fix high amounts of nitrogen when reintroduced to lodgepole pine and western red cedar (gymnosperms). But can this bacterial strain fix nitrogen if introduced into agricultural crops?

**Objectives**

We wanted to determine if this bacterial strain could fix N and promote plant growth while living inside or in rhizosphere of agricultural crops (Corn and Canola).

**Methods**

We inoculated corn and canola seeds with P2b-2R and grew seedlings for 40-60 days. Corn seedlings were harvested 10, 20 and 30 days after inoculation and canola seedlings were harvested after 20, 40 and 60 days after inoculation. Seedlings were evaluated for biological nitrogen fixation and growth promotion.

**Conclusions**

Seedling growth was promoted significantly by inoculation with P2b-2R with an increase of upto 35% in height and 30% in biomass from control. P2b-2R also fixed more than 20% of atmospheric nitrogen while living both inside the plant and in the rhizosphere. These results suggest that this bacterial strain has a broad range of hosts and is successful in N-fixation and growth promotion in agricultural crops.

**FEMS-1416**

**Plant/microbes interactions**

## **EVALUATION OF TEMPERATURE, PH AND CARBON SOURCE IN INDOLE ACETIC ACID PRODUCTION BY YEASTS ISOLATED FROM RIZOSPHERE AND PHYLLOPLANE**

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### **Background**

It is possible to find naturally in rhizosphere and phylloplane some yeasts species capable of producing phytohormones, solubilize phosphates and other minerals, as well as acting as biological control agent. Despite that, scarce information is known about this microorganism group as plant growth promoters.

### **Objectives**

Considering these, the aim of this study was evaluate the production of phytohormone auxin (indole acetic acid (IAA)) by yeasts, with variations of culture conditions.

### **Methods**

The yeasts evaluated were *Rhodotorula mucilaginosa* (2F32) and *Trichosporon asahii* (3S44), isolated from phylloplane and rhizosphere of sugar cane, respectively, in Brazil, São Paulo State. Yeasts were cultivated in Potato Broth medium, with 20 g/L of glucose or sucrose, as carbon source; the cultivation of yeasts was carried out at temperatures 22°C, 25°C or 30°C; pH was modified to 3.0, 4.5 or 6.0. All cultures were analyzed by colorimetric technique with reaction of broth and Salkowsky reagent; the cells count was realized to evaluate the development of yeasts.

### **Conclusions**

The results for *T. asahii* showed that IAA production with sucrose was superior at pH 6.0, and glucose was superior at pH 4.5; these results indicate direct relation between carbon source and pH medium. *Rh. mucilaginosa* presented a high IAA production if compared with *T. asahii* or even with microorganisms related in literature; the higher production (655 µg/ml) was obtained at pH 6.0 and glucose as carbon source. Temperature was not able to influence IAA production, significantly. Variations of culture conditions did not affect the cell growth of yeasts.





**FEMS-1108**

**Plant/microbes interactions**

## **ANALYSIS OF METABOLIC CHANGES IN MESORHIZOBIUM LOTI DURING NODULE MATURATION BY QUANTITATIVE PROTEOMICS**

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### **Background**

Rhizobia are nitrogen-fixing bacteria that establish a symbiotic relationship with leguminous plants. After entering the root cells, rhizobia differentiate into bacteroids within the root nodule of the host plant, and provide the plant with fixed nitrogen. Inside nodule, it has been believed that bacteroids construct optimal environment for nitrogen fixation, but the details are still unknown. We have previously evaluated the intercellular proteome of rhizobia<sup>1</sup>.

### **Objectives**

To understand the mechanism by which rhizobia alter their metabolism to establish nitrogen-fixing symbiotic relationship with hosts, time-course quantitative proteome analysis of *Mesorhizobium loti*, one of the model rhizobia, was performed during the nodule maturation.

### **Methods**

*Lotus japonicus* was inoculated with *M. loti*. Nodules were harvested at 2, 3, and 4 weeks post-inoculation. Bacteroids were extracted from nodules, and their proteins were extracted, digested by trypsin, and labeled by Tandem Mass Tag. The prepared samples were analyzed by LC-MS/MS equipped with a 500 cm-long monolithic silica capillary column<sup>2</sup>.

### **Conclusions**

Using a quantitative proteomics approach, we identified and quantified 537 proteins in *M. loti* bacteroids. The results revealed significant changes in the carbon and amino acid metabolisms upon differentiating into bacteroids. Furthermore, our findings suggest that *M. loti* enters a nitrogen-deficient condition during the early stages of nodule development, and changes to a nitrogen-rich condition in the intermediate stages<sup>3</sup>.

### **Reference**

1) Tatsukami Y. *et al.*, *BMC Microbiol.*, 13, 180 (2013)

- 2) Morisaka H. *et al.*, *AMB Express*, 2, 37 (2012)
- 3) Nambu M. *et al.*, submitted

**FEMS-0770**

**Plant/microbes interactions**

**FUNCTIONAL GENOMICS ANALYSIS OF SALMONELLA-TOMATO INTERACTIONS REVEALS MICROBIAL ADAPTATIONS AND PLANT RESPONSES TO THE INFECTION BY A HUMAN PATHOGEN**

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**Background**

As the number and severity of produce-associated gastroenteritis outbreaks increase, we are coming to recognize that we know little about the ecology of *Salmonella* and enterovirulent *E. coli* outside of their animal hosts.

**Objectives**

The objective of this work was to delineate mechanisms behind interactions of a model human pathogen, *Salmonella enterica*, and its alternate plant host.

**Methods**

TnSeq and RNAseq analyses of *Salmonella* behavior in tomatoes and tomato responses to the colonization by the pathogen were used to reveal key mechanisms of bacterial adaptation to persistence within tomato and plant responses to the colonization by the human pathogen. Expression of individual genes was confirmed by qPCR and Recombinase-based *in vivo* Technology (RIVET).

**Conclusions**

*Salmonella* virulence genes located on the Pathogenicity Islands were not involved in persistence within tomato fruit. We identified important functions for the regulators of capsule synthesis (*yihT*, *rcsB*) in the phenotype. *Salmonella* genes involved in *de novo* synthesis of amino acids were critical to persistence within tomato fruits. *Salmonella* gene expression depended on the host maturity and genotype, for example, RIVET assays confirmed that *cysB* was expressed strongly in a tomato cultivar known to be resistant to a tomato pathogen *Ralstonia solanacearum*, while *fadH* was responsive to the maturity-dependent accumulation of specific fatty acids. Proliferation of the pathogen was restricted in the plant mutants defective in ethylene synthesis and response (*rin*, *Nr*, *nor*). Tomato genes involved in responses to plant pathogens were activated in response to *Salmonella* infection, however, typical responses to infection were only observed in ethylene mutants.

**FEMS-0614**

**Plant/microbes interactions**

## **ENDOPHYTIC COMMUNITIES OF ARABIDOPSIS THALIANA: DOES CULTIVATION SUBSTRATE MATTER?**

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### **Background**

Bacterial endophytes colonize the internal tissues of plants without causing harm. An important subgroup are seed endophytes that are transferred from one generation to the next and that are already present during the early plant growth stages. Several studies suggest that seeds can serve as a vector for beneficial bacteria and that these bacteria could improve plant growth.

### **Objectives**

We want to determine the effect of different cultivation substrates, which are frequently used during bulking of *Arabidopsis thaliana* seeds, on the seed endophytic population of this model plant.

### **Methods**

*Arabidopsis thaliana* was cultivated on sand (bacteria-poor) and on a mixture of sand with potting soil (bacteria-rich). Seeds harvested on both substrates were sown again on the same substrate until the radicle emerged or until there were 3 week-old leaves. Cultivable endophytes were isolated from soil, seeds, radicles and leaves, and identified using 16S rDNA amplification and sequencing. Total bacterial populations were identified by a direct DNA extraction on soil, seeds, radicles and leaves, followed by amplification and 454 pyrosequencing of the V5-V7 hypervariable region of the 16S rDNA.

### **Conclusions**

Despite large differences in the soil bacterial populations, the seed endophytic populations were very similar, which indicates that plants are able to select which bacteria can become seed endophytes. This can impact on the establishment of the endophyte community of the next generation of plants.



**FEMS-1075**

**Plant/microbes interactions**

## **COMPARATIVE EFFECT OF BIO AND NPK FERTILIZERS ON THE GROWTH OF LETTUCE (LACTUCA SATIVA L.)**

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### **Background**

The use of inorganic fertilizers has been established to cause environmental problems.

### **Objectives**

The study investigates the effect of a biofertilizer, *Trichoderma longibrachiatum* NGJ167 and NPK fertilizer on the growth of two varieties of lettuce (Greatlake and Eden).

### **Methods**

The study was carried out in the screenhouse by inoculating soils with mycelial plugs of *T. longibrachiatum* NGJ167 and compared with NPK-fertilizer. Thereafter, *in-vitro* and conventionally propagated lettuce were planted on the treated soils and plant growth parameters observed.

### **Conclusions**

Results showed that the micro-propagated Greatlake lettuce treated with *T. longibrachiatum* had higher plant height (22.23 cm) while the *in-vitro* propagated Greatlake lettuce treated with NPK-fertilizer had mean plant height value of 14.17 cm. The conventional propagated Greatlake and Eden treated with *T. longibrachiatum* had mean plant heights of 17.23 cm and 15.07 cm respectively while the controls had average values of 2.67 cm and 12.4 cm respectively. The average number of leaves of the *in-vitro* propagated Greatlake and Eden lettuce treated with *T. longibrachiatum* (7 and 4 respectively) were higher than those of the NPK-fertilized Greatlake and Eden lettuce (6 and 5 respectively). The result of the fresh biomass revealed that the conventional propagated Eden lettuce treated with *T. longibrachiatum* had an average value of 19.77 g while the NPK-fertilized plants had a mean of 15 g. The implication of these results showed that *T. longibrachiatum* NGJ167 was better than NPK fertilizer with respect to the growth of lettuce. The study concluded that the use of *T. longibrachiatum* improved the growth of lettuce.

**FEMS-1150**

**Plant/microbes interactions**

**MOLECULAR CHAPERONES OF THE TYPE III SECRETION SYSTEM ARE NOT REQUIRED FOR SECRETION OF THEIR PARTNER SUBSTRATES, BUT CAN BLOCK EFFECTOR TRANSPORT IN PECTOBACTERIUM CAROTOVORUM**

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**Background**

*Pectobacterium carotovorum* (*Pca*) is a plant pathogenic soft rot bacterium that can use the type III secretion system (T3SS) to suppress host defence. T3SS is utilized by many Gram negative bacterial pathogens to inject bacterial virulence proteins into eukaryotic cells, where they can manipulate host cell processes to pathogen advantage. T3SS chaperones are involved in protein transport, but their exact function is still unclear in many cases. In *Pca* two potential T3SS chaperones are coded for by the divergent transcriptional units, *dspEF* and *hrpWshcW*. Chaperone function was suggested for the DspF protein in related bacterium *Erwinia amylovora*, but not in *Pca*, while the ShcW protein was not studied before.

**Objectives**

Determine the role of DspF and ShcW in T3SS-dependent protein transport in *Pca*.

**Methods**

PCR, molecular cloning, directed mutagenesis, DNA sequencing, far-western blotting, two-hybrid screening.

**Conclusions**

We have found no evidence for chaperone requirement for stability or secretion of the DspE and HrpW effector proteins. On the other hand, introduction of additional copies of the *dspF* or *shcW* genes into *Pca* cells blocks their ability to induce hypersensitive response on nonhost plants. This phenotype is caused by a severe defect in translocation of effectors into plant cells. Translocation of the DspE-Cya fusion appeared to be completely blocked while translocation of AvrPto-Cya – reduced from *Pca* cells carrying *dspF* on a plasmid. Thus, additional molecules of T3SS chaperones can block effector translocation, while their native amounts are not required for secretion/translocation. This suggests that the main function of the DspF and ShcW chaperones may be to repress premature effector transport.

**FEMS-2990**

**Plant/microbes interactions**

**CYCLIC  $\beta$ -(1,2)-GLUCAN FROM XANTHOMONAS CAMPESTRIS PV. CAMPESTRIS SUPPRESSES ARABIDOPSIS THALIANA IMMUNITY BY MODULATING FLAGELLIN INDUCED DEFENSE.**

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**Background**

Plants are constantly exposed to microbes, such as bacteria, fungi or viruses and most plants are resistant to most microbes. To be successful, a pathogen must overcome constitutive defenses or suppress induced defenses. Our group previously demonstrated that cyclic  $\beta$ -(1,2)-glucan, a polysaccharide synthesized by *Xanthomonas campestris pv. campestris*, suppresses the accumulation of callose deposition, as well as the local and systemic expression of PR1, a defense-related gene associated with salicylic acid responses (The Plant Cell. 2007: 2077-2089).

**Objectives**

To gain knowledge about the mechanism of cyclic glucan in the modulation of plant defense

**Methods**

We performed *Xanthomonas campestris pv. campestris* infection assays on *A. thaliana* leaves pretreated with flagellin (flg22) and purified cyclic glucan or both. Measurement of WRKY22, WRKY33 and MPK3 expression was done by RT-PCR and binding assays was made using *Arabidopsis* membranes and labeled <sup>14</sup>C-cyclic glucans.

**Conclusions**

We observed that cyclic glucans of *X. campestris* suppresses partially the immunity elicited by flg22 in *Arabidopsis thaliana*. In addition, this compound downregulates WRKY22, WRKY33 and MPK3 expression, early defense genes induced by this elicitor. In order to get further insights into how CG modulates flg22 responses, we performed binding assays with *Arabidopsis* membranes and labeled <sup>14</sup>C-CG. The radiolabeled glucan, <sup>14</sup>C-CG, bound specifically to wild-type plant extracts, suggesting the existence of direct interaction between CG and an still unknown *Arabidopsis* receptor. The present results shed light on the mechanism by which *Xcc* CG hijacks, at least partially, plant immune response.



## FEMS-3023

### Plant/microbes interactions

#### **XBMR, A NOVEL TRANSCRIPTION FACTOR REGULATES CHEMOTAXIS, BIOFILM FORMATION AND VIRULENCE IN XANTHOMONAS CITRI SUBSP. CITRI**

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#### **Background**

Citrus canker is one of the most important and aggressive bacterial diseases of citrus trees. The causal agent of this disease is *Xanthomonas citri* subsp. *citri* (Xcc). Biofilm formation on citrus leaves plays an important role in epiphytic survival of Xcc. Previous work from our laboratory described a genetic screen for biofilm formation-defective mutants in Xcc, this work identified that a mutant with a transposon insertion in XAC3733 (*xbmR*) had significantly reduced attachment to a polystyrene surface (Microbiology. 2013: 159, 1911–1919).

#### **Objectives**

To gain knowledge about the regulatory mechanisms of Xcc infection

#### **Methods**

For in vitro analysis of biofilm formation, we used confocal laser scanning microscopy. For pathogenicity assays grapefruits leaves was used as the host plant for Xcc. Gene expression levels was obtained by qRT-PCR. Intracellular alteration in c-di-GMP was achieved by exogenous expression of either the diguanylate cyclase WspR19 from *P. fluorescens* or the c-di-GMP phosphodiesterase PA2567 from *P. aeruginosa*.

#### **Conclusions**

A knock-out of *xbmR* led to a substantial downregulation of *fliA*, which encodes a  $\sigma^{28}$  transcription factor, as well as *fliC* and XAC0350 which are potential member of the  $\sigma^{28}$  regulon. XAC0350 encodes an HD-GYP domain cyclic di-GMP phosphodiesterase. These findings suggest that XbmR is a key regulator of flagellar-

dependent motility and chemotaxis exerting its action through a regulatory pathway that involves FliA and c-di-GMP.

**FEMS-1036**

**Pollutant degradation**

## **MICROBIAL RESOURCES TO BIODEGRADE COMPLEX TAR AFTER STEAM GASIFICATION PROCESSES**

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### **Background**

Biomasses gasification produces toxic compounds (tar), mainly composed by polycyclic aromatic hydrocarbons (PAH). Up to now the problem of the tar disposal is not solved. Bioremediation of tar with a mixed microbial consortium could be a highly promising and innovative approach for the handling of this by-product.

### **Objectives**

The aim of this study was to assess the tar-degradation process in a laboratory-scale microcosm.

### **Methods**

Triplicate tar-containing microcosms (PAH 13,904 mg/L) were incubated for 20 days with a microbial consortium selected from pinewood and polluted soils. After 20 days HPLC and GC/MS showed a significant reduction of all the PAH compounds to about 150 mg/L whereas PAH concentration in the negative controls was about 8,400 mg/L. Reliability of distinct replicated microcosms was very high since microbial communities showed 97% of similarity. Automated fingerprintings and ribosomal gene pyrosequencing showed, at first, a noticeable lag-phase (0-2 days), where fungi were the leading part of the community. A second phase was evident between 3 and 10 days of degradation with specific bacterial genera. After 15 days, a stable phase with 24 bacteria genera and only one fungal genus was detected. Overall, a total of 59 bacterial and 22 fungal genera participated in the degradation.

### **Conclusions**

Bacterial species grew according to a “cross-feeding” behavior. Natural and polluted soils proved to successfully provide valuable microbial taxa for innovative services such as tar degradation.



**FEMS-1716**

**Pollutant degradation**

**COMPARISON OF BACTERIOPLANKTON COMMUNITIES AND DIESEL BIOREMEDIATION ABILITY OF NATIVE BACTERIA FROM DIFFERENT MEDITERRANEAN TOURIST PORTS**

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**Background**

Tourist ports are subjected to seasonal impacts determining high pollution by petroleum hydrocarbons. Bioremediation is an emerging clean-up technique based on the addition of amendments or microorganisms able to stimulate the degradation activity of the autochthonous bacteria.

**Objectives**

This study was carried out within MAPMED, a multidisciplinary project aimed to improve the environmental sustainability of tourist ports in the Mediterranean Sea with regard to hydrocarbon pollution. Cagliari (Italy), El Kantaoui (Tunisia), and Heraklion (Greece) were selected as case study sites. The present study aims to compare bacterioplankton, hydrocarbon degraders, and the effects of different bioremediation additives on the autochthonous communities among the selected sites.

**Methods**

The structures of bacterioplankton were compared by 16S rRNA T-RFLP among sites, sections with different usage and seasons (winter, beginning and end of the tourist season). Cultivable hydrocarbon degraders were characterized regarding phylogenetic position and catabolic abilities. Different bioremediation treatments (biostimulation with nutrients and bioaugmentation with selected strains) were tested in seawater microcosms supplemented with diesel. The monitoring parameters are the titles of heterotrophs and diesel degraders, and the hydrocarbon removal efficiency. At the end of treatments, the bacterioplankton communities are compared by 16S rRNA gene analysis.

**Conclusions**

The structure analysis highlights a clear seasonal variation in bacterioplankton and differentiates Heraklion communities from those found in Cagliari and El Kantaoui. Degraders exhibiting different ecological strategies dominate the three communities, copiotrophics in Cagliari and hydrocarbonoclastic bacteria in El Kantaoui and

Heraklion. Comparison of the effects of different bioremediation treatments is currently in progress.

FEMS-2733

## Pollutant degradation

### EXTRACELLULAR DNA AS BIOSCAFFOLD FOR THE SYNTHESIS OF SUPERIOR PALLADIUM(0) NANOCATALYSTS HIGHLY ACTIVE IN THE DETOXIFICATION OF PERSISTENT ORGANOHALOGEN POLLUTANTS

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#### Background

In a search for new aqueous-phase systems for catalyzing reactions of environmental and industrial importance, we prepared novel palladium(0) nanocatalysts in the presence of *Shewanella oneidensis* strains. Especially a nuclease-deficient mutant of *Shewanella oneidensis* MR-1 showed an increased tolerance against elevated heavy-metal stress, obviously as a result of high levels of extracellular DNA (eDNA) and additional adaptive response mechanisms.

#### Objectives

The main objective of our study was to assess whether eDNA is able to protect *S. oneidensis* cells from toxic Pd(II) ions and can simultaneously serve as a scaffold for nanocatalyst synthesis.

#### Methods

With eDNA as nucleation site, it was possible to retrieve superior nanocatalysts, bearing small sizes, a monodisperse size distribution and low aggregation behaviour which are important prerequisites for high catalytic activity. Furthermore, in comparison to catalysts produced by the *Shewanella oneidensis* wildtype, the nanocatalysts synthesized by the mutant were more resistant against catalyst transformation, deactivation and poisoning. The catalytic properties of the palladium(0) nanocatalysts were tested using a newly developed real-time PTR-ToF-MS analytical technique, which is able to follow the nanocatalyst-mediated detoxification of highly-persistent anthropogenic pollutants at environmentally relevant concentrations. The palladium(0) nanocatalysts synthesized in the presence of the nuclease-deficient mutant of *Shewanella oneidensis* MR-1 showed a high catalytic activity for the dehalogenation of hexachlorobenzene and triclosan.

#### Conclusions

Further tests with 'old' and emerging halogenated persistent organic pollutants are under way and will demonstrate the high potential of Pd(0) nanocatalysts for clean-up of drinking water, aquifers and specific industrial effluents, as well as the suitability of PTR-ToF-MS to assess the respective transformation and detoxification reactions.





**FEMS-0588**

**Pollutant degradation**

**COMPARATIVE STUDY OF THE CAPACITY OF CARRIERS: ANOXKALDNE K1, POLYPROPYLENE PADS AND GRANULAR CORKSORB TO FORM HYDROCARBON-DEGRADING BIOFILMS AND REMOVE HYDROCARBONS FROM DIESEL POLLUTED WATER**

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**Background**

Hydrocarbons are a widely distributed pollutant that reaches the environment from multiple sources and involve significant risks to the environment that must be minimized

**Objectives**

To design a new treatment system for hydrocarbon contaminated water based on biofilm technology, we studied three different carriers 1) AnoxKaldnes™ (a plastic carrier). 2) Polypropylene pads, these are oil absorbent pads composed of 100% meltblown polypropylene that absorb oil only and not water. 3) Granular thermal treated hydrophobic cork which absorbs oil and solvents without absorbing water.

**Methods**

Using 1L bioreactor with 400 ml of water and 200 ml of carrier, kinetics of growth and adherence to carriers were determined by enumeration of viable cells. Development of biofilm was visualized by scanning electron microscopy. TPH, n-alkanes, branched-alkanes and naphthalene removal were determined by GC/MS at the beginning and at the end of the assays. *Pseudoalteromonas elyakovii* strain W18 was inoculated to study biofilm formation. Waters with and without 1% (v/v) diesel, were used to analyze the characteristics of formed biofilm and the efficiency of hydrocarbon removal by indigenous microorganisms.

**Conclusions**

No inhibitory effect of carriers was observed, even in bioreactors with high hydrocarbon contamination. SEM analyses showed that Polypropylene pads and Granular CorkSorb allow microbial biofilm formation. GC/SM analyses showed that Polypropylene pads not only enhance microbial growth but also retain high amount of hydrocarbons.

*Acknowledge:* This research has been supported by grant of Compañía Logística de Hidrocarburos CLH, S.A.

## **FEMS-1929**

### **Pollutant degradation**

#### **NOVEL ANAEROBE OBTAINED FROM A HEXADECANE-DEGRADING CONSORTIUM**

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#### **Background**

Aliphatic hydrocarbons (AHC) are abundant in crude oil and fuels, and are frequent contaminants of water, soil and sediments. There is potential for AHC bioremediation using sulfate as electron acceptor, due to its abundance in marine environments and natural presence in soils and groundwater.

#### **Objectives**

In this work sulfate-reducing anaerobic microorganisms involved in AHC biodegradation were studied.

#### **Methods**

Anaerobic sludge was incubated at 37°C with hexadecane (1mM) and sulfate (20mM) in serum vials. Cultures were successively transferred to fresh medium until a stable enrichment was obtained (monitored by microscopy and PCR-DGGE of 16S rRNA gene). For isolation of AHC-degrading bacteria, serial dilutions and successive transfers are now running using palmitate (1mM) as an easier substrate.

#### **Conclusions**

Cultures growing on palmitate show two main bacterial cell types: a rod-shaped bacterium closely related to *Desulfomonile limimaris* (94% identity) was predominant in the first 30 days of incubation, when 83% of the added palmitate was degraded coupled to 4 mM sulfate reduction (suggesting stoichiometric palmitate conversion to acetate); and an oval-shaped bacterium related to *Desulforhabdus amnigena* (99% identity) that mainly developed when incubations were extended and a total of 11.5 mM sulfate was reduced. Growth of *Desulforhabdus* was stimulated when incubated with acetate. The role of the *Desulfomonile* in AHC degradation will be further discussed in the presentation, as well as its halorespiring ability, a characteristic of

the Desulfomonile genera. Further characterization of this novel bacterium is important due to its high potential for bioremediation of hydrocarbons, fats and halogenated pollutants.

**FEMS-1181**

**Pollutant degradation**

## **APPLICATION OF HIGHLY EFFECTIVE MICROBIAL DEGRADERS OF OILS AND FATS IN WASTEWATERS DECONTAMINATION**

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### **Background**

Disposal of municipal sewage and industrial effluents remains a top priority challenge. Annual discharge of wastewaters containing lipid substances in Belarus reaches 10-12 mln ton. It is recommended to utilize high-efficient microbial strains decomposing oils and fats for their remediation.

### **Objectives**

Searching and examination of microorganisms-degraders of lipid compounds.

### **Methods**

Isolation of microbial strains was carried out by enrichment culture technique. Lipase activity was evaluated by Ota-Yamada method. Dynamics of lipid dissimilation was monitored on model water comprising fats (lard, dairy) and oils (sunflower and olive) in concentration 1000 mg/l as the sole carbon sources.

### **Conclusions**

Four superactive bacterial strains *Rhodococcus ruber* 2B, *Rhodococcus* sp. R1-3FN, *Bacillus subtilis* 6/2-APF1, *Pseudomonas putida* 10AP – degraders of fatty substrates showing lipase activity levels in the range 0.65-0.70 u/mg protein were derived by adaptive selection method. It was found that the selected strains were capable to consume 83.4-94.2 % of animal fats and 82.3-90.4 % of vegetable oils in 7 days. Nutrient medium composition was defined and optimized in terms of nitrogen, potassium, phosphorus and carbon sources. It allowed to attain maximum biomass concentrations (3.0-4.8 g/l by 48 h of fermentation) with elevated lipase activity.

Based on the obtained data technology of manufacturing microbial preparation to accelerate disposal of lipid pollutants in wastewaters was developed.

Introduction of new microbial product into biodecontamination scheme promoted 15.9 % decline in chemical oxygen demand, increased degree of lipid removal by 59 % and improved quality of activated sludge.

**FEMS-1717**

**Pollutant degradation**

**APPLICATION OF CARBON STABLE ISOTOPE ANALYSIS (CSIA) TO INVESTIGATE BIODEGRADATION AND DIRECT PHOTOLYSIS OF ANTIBIOTIC SULFAMETHOXAZOLE**

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**Background**

Sulfamethoxazole (SMX) represents sulfonamide antibiotics (SAs) widely used in veterinary and human medicine. SMX is incompletely metabolized and enters the environment with wastewater discharge, animal manure and aquaculture. SMX is frequently detected in wastewater treatment plants, surface and ground water. In the environment it undergoes different degradation processes, such as biodegradation and photodegradation.

Understanding of SA's removal routes is crucial for an assessment of their environmental impact. Compound specific stable isotope analysis (CSIA) can provide additional information on the organic contaminants' transformation processes in complex environments. In CSIA, the changes in isotope composition of the parent compound are monitored during (bio)transformation processes and the isotope enrichment of the investigated contaminant provides an evidence for its (bio)degradation without a need of metabolite analysis.

**Objectives**

This research aims to evaluate the applicability of CSIA for the assessment of SMX transformation pathways. Therefore, the isotope fractionation of SMX during biotic degradation by *Microbacterium* sp. strain BR1 and abiotic transformation via direct photolysis was determined.

**Methods**

Bio- and photodegradation assays; HPLC; LC-IRMS; quantification of enrichment factors

**Conclusions**

A significant difference in isotope fractionation during biotic and abiotic SMX decomposition was observed, showing that CSIA has a potential for distinguishing these two degradation processes. Isotope fractionation during direct photolysis was variable and depended on pH of the solution.

This work shows a new application for CSIA - monitoring of sinks, sources and environmental behaviour of water-soluble contaminants, among which pharmaceuticals are of particular concern.

## **FEMS-2550**

### **Pollutant degradation**

#### **BIOREMEDIATION OF DRILL CUTTINGS BY HALOPHILIC BACTERIA**

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#### **Background**

Exploration and drilling of oil requires the use of drilling fluid to lubricate and cool the drill bit. Oil based muds (OBMs) containing diesel, are preferred drilling fluids. After mixing with drill cuttings, OBMs form complex industrial wastes containing hydrocarbons and brine. Bioremediation is a promising alternative to physicochemical methods for cleaning up drilling wastes.

#### **Objectives**

The goal of this study was to isolate halophilic bacterial consortium capable of degrading diesel oil in drilling muds and evaluating their applicability in the lab scale drill cuttings microcosm.

#### **Methods**

Between 10 microbial consortia isolated from different saline environments, the best consortium was isolated from Qom sample which grew very well on Bushnell Haas medium containing diesel with the bacterial count about  $1.9 \times 10^{15}$  CFU/ml after 96h of incubation. For evaluating the applicability of the consortium in bioremediation of drill cuttings, it was mixed by 50% of fine sands or biological active soil in the lab scale microcosms and the effect of consortium inoculation was compared with corresponding un-inoculated control for three month.

#### **Conclusions**

The Measurement of the amount of remaining pollutant (TPH) after 3 month showed that the most degradation rate (40%) belonged to the microcosm containing 50% fine sand and consortia inoculation. Sequencing of 16S rRNA gene revealed that the dominant strain of the consortium belonged to the *Dietzia* genus. It could be concluded that bioaugmentation is useful for remediation of drill cuttings when the cuttings mixed with fine sand which has a weak microbial flora and allowing the better growth of inoculated consortium.





**FEMS-1548**

**Pollutant degradation**

**ISOLATION AND CHARACTERIZATION OF CONFLUENTIMICROBIUM SP. NS6, A NEW NAPHTHALENE-DEGRADING BACTERIUM, ISOLATED FROM AN OIL-CONTAMINATED TIDAL FLAT**

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**Background**

Polycyclic aromatic hydrocarbons (PAHs) are great environmental concerns because of their persistence, toxicity, and carcinogenicity. Many studies for bacteria capable of PAH degradation have been reported in seawater, but PAH-biodegrading bacteria in tidal flats have been rarely explored.

**Objectives**

The purpose of this study was to isolate strains NS6 as a novel PAH degrader capable of PAH degradation in tidal flats and characterize its physiological and PAH-biodegrading properties.

**Methods**

We established a slurry type enrichment system based on tidal flat sediment and seawater using naphthalene as a sole carbon and energy source. The enrichment culture was spread on marine agar (MA) and incubated at 25°C for 2 days. Colonies grown on MA were characterized phylogenetically based on their 16S rRNA gene sequences and the naphthalene degradation abilities were evaluated in seawater, tidal flat slurry, and ONR7a. Finally, a strain showing a good naphthalene degradation property was isolated and its PAH degradation properties and genes were characterized.

**Conclusions**

A naphthalene degrading bacterium, designated strain NS6, was isolated from the slurry type enrichment and classified into a member of *Confluentimicrobium* based on its 16S rRNA gene sequence. *Confluentimicrobium* sp. NS6 degraded PAH compounds quickly in all tested media. Naphthalene dioxygenase gene of *Confluentimicrobium* sp. NS6 was successfully amplified by only NDO 201 primer set, which suggested that strain NS6 may metabolize naphthalene via a *nah* catabolic pathway alike *Rhodococcus*. In addition, the PAH degradation properties, physiologies, and PAH degradation gene structure of strain NS6 will be investigated and discussed in detail in the poster section.

**FEMS-1249**

**Pollutant degradation**

## **OXIDATION OF AROMATIC COMPOUNDS BY VERSATILE PEROXIDASE (VP1) OF PLEUROTUS OSTREATUS UNDER MN<sup>2+</sup>-DEFICIENCY**

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### **Background**

The use of white rot fungi such as *Pleurotus ostreatus* and their lignin-modifying enzymes has become an effective treatment for various organic soil and water pollutants. The manganese peroxidase gene family (*mnps*) is a major part of the ligninolytic system of *P. ostreatus*. This gene family is comprised of nine genes encoding short manganese peroxidases (short-MnPs) or versatile peroxidases (VPs). The VPs contain unique active sites which are responsible of direct oxidation (in the absence of Mn<sup>2+</sup>) of various aromatic compounds.

### **Objectives**

Here we study the oxidation mechanisms of aromatic compounds by VP1 of *P.ostreatus*.

### **Methods**

We show that in Mn<sup>2+</sup>-deficient GP medium *vp1* (encoding VP1) has a key and non-redundant function. We used the azo-dye Orange II (OII) as a model contaminate, its decolorization occurs only during the idiophase where the abundance transcripts of *mnps* indicate that *vp1* is the predominantly expressed and a  $\Delta vp1$  strain showed a drastic reduction in the decolorization. Three degradation metabolites were identified by LC-MS indicating both asymmetric and symmetric enzymatic cleavage of the azo bond. The presence of asymmetric cleavage diminishes the toxicity level of the degradation products. To better understand mechanisms of degradation and detoxification we purified and characterized VP1. The purified enzyme degraded 60% of OII (50μM) within the first min and continued up 90% within 6 min.

### **Conclusions**

The non-specific oxidation properties of VP1 and its unique ability to degrade organic compound in the absence of Mn<sup>2+</sup> suggest that it may have potential applications for treatment of contaminated water.

**FEMS-0879**

**Pollutant degradation**

**FUNGAL DEGRADATION OF POLYSTYRENE AND ITS ANALOGUE  
POLYSTYRENE SULFONATE - IMPLICATIONS ON PLASTICS WASTE  
BIOREMEDIATION**

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**Background**

Accumulation of synthetic polymers in the environment is cause for concern, as they have been associated with various negative effects on ecosystem health, especially marine systems. Knowledge on biodegradation pathways of conventional polymers is very limited, but it is known that these compounds are hardly biodegradable.

**Objectives**

Here, we investigated whether fungi causing white and brown rot of wood are capable of degrading the widespread polymer polystyrene (PS) as well as its water-soluble analogue polystyrene sulfonate (PSS). Their well-known extracellular oxidation capabilities made them promising candidates for the degradation of these recalcitrant substrates.

**Methods**

PSS analysis by size exclusion chromatography revealed that it was practically inert to white-rot fungi, but vulnerable to various brown-rot strains, which caused strong depolymerisation leading to molecular mass reductions of more than 90 %. Detailed investigations pointed to Fenton reactions driven by fungal hydroquinones as agents of depolymerisation. Solid PS films were significantly more recalcitrant, as no changes in their bulk properties were observable after long periods of fungal treatment. Indications of biodegradation only came from surface property measurements, which revealed increased hydrophilicity through slightly decreased water contact angles. Investigations by X-ray photoelectron spectroscopy revealed that surface oxygen content had increased significantly in fungi-treated samples.

**Conclusions**

These results demonstrate that biodegradation of recalcitrant synthetic polymers is, in principle, possible. However, the different observations between PS and PSS indicate that biological attack on the solid polymer proceeds extremely slowly, raising questions about the feasibility of PS waste biodegradation.

## FEMS-3122

### Pollutant degradation

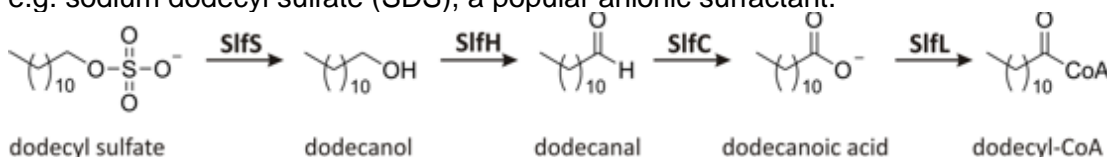
#### FUNCTIONAL ANALYSIS OF A PLASMID-BORNE ALKYL SULFATE DEGRADATION MODULE OF *PSYCHROBACTER* SP.

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#### Background

Plasmid pP62BP1 (34.5 kb), harboured by strain *Psychrobacter* sp. DAB\_AL62B isolated from ornithogenic deposits in Spitsbergen (Svalbard Archipelago, Norway), was found to carry a phenotypic module, named SLF, which consists of four open reading frames (*slfCHSL*) encoding putative catabolic enzymes and a gene (*slfR*) for transcriptional regulator of AraC/XylS family. Based on the comparative *in silico* analyses, the module was predicted to take part in the metabolism of alkyl sulfates, e.g. sodium dodecyl sulfate (SDS), a popular anionic surfactant.



#### Objectives

The aim of our work was to perform a functional characterization of the SLF module both in native host and in other bacterial strains as well as to elucidate the regulatory mechanism of the *slfRCHSL* genes expression.

#### Methods

Qualitative and quantitative SDS degradation assays were designed based on the properties of Stains-All, a carbocyanine dye. Promoter strength was examined in  $\beta$ -galactosidase activity assays. Transcriptional organization of the module was characterized with the use of reverse transcription PCR.

#### Conclusions

Growth experiments revealed that 0.17 mM concentration of SDS in the medium is sufficient to exert bacteriostatic effect on several tested *Psychrobacter* spp. strains. Nonetheless, the pP62BP1 containing strain was capable of the complete SDS degradation in these conditions. The cloning of the whole SLF module and its *slfR*-deficient derivative into *Escherichia coli* TG1 resulted in obtaining transformants able to degrade SDS. The molecular studies showed that the activity of the *slfCHSL* operon is dependent on SlfR protein which acts as a negative transcriptional regulator.

## **FEMS-1870**

### **Pollutant degradation**

#### **MOLINATE BIOREMEDIATION STRATEGY USING A MICROENCAPSULATION APPROACH**

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#### **Background**

Molinate is a thiocarbamate herbicide used in rice cultivation to control grass weeds, however its use had led to environmental contamination, being critical the establishment of remediation procedures. A mixed culture has been described as being able to mineralize molinate as the sole source of carbon, nitrogen and energy [1, 2], primarily, by the activity of molinate hydrolase (MolA) [3], encoded by *Gulosibacter molinativorax* ON4<sup>T</sup>. Previous studies, using soil microcosms, have demonstrated that a bioaugmentation strategy using the mixed culture was a feasible approach to bioremediate paddy soils contaminated with molinate [4].

#### **Objectives**

To improve the bioremediation strategy, we aimed at microencapsulating ON4<sup>T</sup> cells or MolA, using a spray-drying process that uses biopolymers as encapsulating agent [5].

#### **Methods**

Several encapsulating agents (sodium and calcium alginate, arabic gum, chitosan and modified chitosan) were used. The spray-drying product yield was about 50% for all the agents. The metabolic activity of the microencapsulated cells was proven by a qualitative colorimetric method using a redox dye (tetrazolium violet).

#### **Conclusions**

There was a linear correlation between the amount of encapsulated cells and the colour development intensity. The feasibility of using optimized microencapsulated biomaterial will be tested in contaminated soil and water using a microcosm approach.

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**FEMS-1048**

**Pollutant degradation**

## **INACTIVATION OF PSEUDOMONAS AERUGINOSA BY ZINC OXIDE NANOPARTICLES IN AQUEOUS SOLUTION**

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### **Background**

With the emergence of microorganisms resistant to multiple antimicrobial agents there is increased request for promotion of disinfection methods.

### **Objectives**

Since ZnO nanoparticles (ZnO-NPs) exhibit strong antibacterial activities on a broad spectrum of bacteria the aim of this study was to evaluate the antimicrobial activity of ZnO-NPs against *pseudomonas aeruginosa* as a model for gram-negative bacteria.

### **Methods**

Muller Hinton broth was used as a growing medium for *pseudomonas aeruginosa*. Photocatalytic experiment was carried out in a laboratory-scale batch reactor with low pressure ultraviolet irradiation (380 nm). Different experimental parameters such as amount of ZnO-NPs, contact time, inorganic and organic substances and pH on photocatalytic inactivation of *pseudomonas aeruginosa* cells have been studied. An initial *pseudomonas aeruginosa* concentration of 10<sup>8</sup> CFU/mL was used for all experiments.

### **Conclusions**

Result showed that, almost all the initial *pseudomonas aeruginosa* cell (10<sup>8</sup> CFU/ml) was inactivated in 60 min in the presence of 2 g/l ZnO-NPs. Photocatalytic inactivation of bacteria was found to follow first order kinetics. The initial pH of the water did not play an important role on the inactivation rate within a range of 6–8 pH units. The amount of photocatalyst also plays an important role in photocatalytic inactivation rate. As the result showed increasing the photocatalyst amount provided more rapid inactivation. Addition of some inorganic ions to the suspension affects the sensitivity of *pseudomonas aeruginosa* and caused to retard the inactivation rates.

Since the sensitivity of *pseudomonas aeruginosa* to photocatalytic treatment was fairly good, it is therefore, recommended to use this nano-particle for water treatment.

**FEMS-3102**

**Pollutant degradation**

**ADAPTATION TO AROMATIC HYDROCARBONS INVOLVES A NEW HYBRID TWO-COMPONENT REGULATORY SYSTEM IN AZOARCUS SP. CIB**

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**Background**

The catabolic processes performed in anaerobiosis are becoming of great environmental interest because many anoxic ecosystems are frequently contaminated with aromatic compounds. The facultative anaerobe  $\beta$ -proteobacterium *Azoarcus* sp.CIB has been used as model system to study basic aspects on the biochemistry and genetics underlying the anaerobic metabolism of several aromatic compounds. Within the operon responsible for the anaerobic degradation of toluene, we identified an orphan gene, *tolR*, without homologues. The TolR protein shows an atypical architecture which corresponds to a new hybrid two-component system (HTCS), where the sensor histidine-kinase and the c-di-GMP phosphodiesterase-containing response regulator (RR) components are fused in a single polypeptide.

**Objectives**

The main objective was to demonstrate that TolR constitutes a new HTCS involved in anaerobic adaptation to aromatic hydrocarbons in *Azoarcus* sp.CIB

**Methods**

An *in vivo* approach for confirming the c-di-GMP phosphodiesterase activity of the *tolR* gene was done using a *lacZ*-based *P. aeruginosa* reporter strain. *In vitro* approaches by using purified TolR and truncated forms were used to demonstrate toluene-induced autokinase and transphosphorylation activity. Transcriptomic studies were done to identify genes involved in the TolR-mediated response to aromatic hydrocarbons.

**Conclusions**

TolR encodes a unique HTCS whose RR is not a transcriptional regulator but a c-di-GMP phosphodiesterase. We demonstrate for the first time the effector-dependent induction of the intramolecular phosphorelay in HTCS. Transcriptomic studies confirmed that TolR is involved in a signalling network that controls the morphological, metabolic and stress response programs. The toluene-dependent control of c-di-GMP levels represents an unprecedented mechanism of bacterial adaptation to aromatic hydrocarbons.

**FEMS-1159**

**Pollutant degradation**

## **BIODEGRADATION POTENTIAL OF *PENICILLIUM* SP. ISOLATED FROM SOUTH CHINA SEA**

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### **Background**

Some halophiles fungal genera were found from sea sediment samples are potentially play an important role in nutrient regeneration cycles as decomposers.

### **Objectives**

In this study, three genera of microfungi derived from South China Sea were isolated as *Penicillium*, *Aspergillus* and *Talaromyces* in order to identify the fungal saprotrophic properties.

### **Methods**

For the first stage of investigation by using dermatophyte test medium (DTM), the results showed that only the ascomycete *Penicillium* sp. highly potential as saprophytic microfungi. This fungus was used to observe their early thallus development *in vitro* and to determine a degradation potential of the textile dyes Trypan blue (TB), Methyl orange (MO), Sudan III (S3), Congo red (CR) and Bromocresolgreen (BG) in broth culture.

### **Conclusions**

*Penicillium* sp. displays a distinctive morphological growth, produced an orange pigment in all different media as an additional features of the strain. In contrast, there were significant differences in physiological tests of *Penicillium* sp. such as thermophilic osmophilic, pH, salinity, xerotolerant and nitrogen stress. In the presence of textile dyes in liquid media was able decolorized by *Penicillium* sp. Thus, this study shows that halophilic *Penicillium* sp. from sediment of South China Sea is hereby reported for the first time and will be used as bio-indicator of water pollution and also in biological wastewater treatment. In addition this investigation also demonstrated the need of extra and intracellular enzymes associated to decolorization and biodegradation assays.

## FEMS-1784

### Pollutant degradation

#### STUDIES ON HYDROGEN SULFIDE OXIDASE FROM PSEUDOMONAS SP.

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#### Background

Biogas resultant from the anaerobic treatment of sludge is composed mainly of CH<sub>4</sub> and CO<sub>2</sub>. H<sub>2</sub>S, a minor component, is of particular concern due to its corrosive and environmentally hazardous properties. Some microorganisms are able to perform enzymatic oxidation of H<sub>2</sub>S to sulfur and/or sulfate. Biological treatment using these microorganisms can be an alternative to physical/chemical processes, avoiding the production of secondary waste.

#### Objectives

This work aims at the characterization of enzyme(s) responsible for H<sub>2</sub>S oxidation in *Pseudomonas* sp.

#### Methods

Isolates A9, B9 and C1, identified as *Pseudomonas* spp., were isolated through enrichment from wastewater treatment plant deodorant bioreactor supplied with H<sub>2</sub>S streams. These strains were screened for the presence of genes responsible for H<sub>2</sub>S oxidation by PCR using degenerated primers for group II *sulfide quinone:oxidoreductase*. Fragments with the expected size were purified from the gel and subjected to sequencing. Translated sequences were compared using the BLAST software. Total protein profiles were screened by SDS-PAGE, before and after the addition of H<sub>2</sub>S. Activity tests were conducted using crude cell extract as catalyst and measuring the formation of sulfate as the reaction product.

#### Conclusions

The amplified fragment from isolate A9 showed 51% identity with an *oxidoreductase* from *Pseudomonas* sp. M1, indicating that this bacterium has the machinery required to the desired activity. The protein profile of the strains when grown in the presence H<sub>2</sub>S differ from the profile of the same strain grown without sulfide.

**FEMS-1760**

**Pollutant degradation**

## **NOVEL POLYESTERASES INVOLVED IN ANAEROBIC DEGRADATION OF SYNTHETIC POLYMERS BY CLOSTRIDIUM SPP.**

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### **Background**

The biodegradability of PBAT (poly(butylene adipate-co-butylene terephthalate)) under aerobic conditions has been examined in several studies. However, there is considerably less known about PBAT biodegradation in anaerobic environments.

### **Objectives**

Amongst others *Clostridium* species are known to be typically present during naturally occurring anaerobic degradation processes of polymers (e.g. in biogas plants). Hence, various *Clostridium* species were in-silico screened for esterases that are possibly active on synthetic polyesters like PBAT.

### **Methods**

First, a selection of identified hydrolases from *Clostridium* species were successfully expressed in *E. coli* BL21-Gold(DE3). The activity of these esterases was confirmed on the soluble standard substrate *p*-nitrophenyl butyrate as well as on the PBAT polymer. All esterases were characterized in detail including the determination of their crystal structure or modeling of the proteins. The crystal structure of one promising esterase revealed the presence of a metal ion that lies deep beneath the protein surface. Furthermore, *Clostridium* cultures were tested for their ability to express these active and naturally occurring enzymes in situ and degrade the PBAT polymer.

## Conclusions

We were able to prove that the novel hydrolases from *C. botulinum* and *C. hathewayi* hydrolyze the aliphatic-aromatic polyester PBAT. These enzymes are promising candidates for industrial applications or pollutant degradation. Furthermore the study provides information about these anaerobic strains and their naturally occurring enzymes.

**FEMS-1756**

**Pollutant degradation**

## **USE AND IMPACT OF $\text{MnO}_2$ MICROPARTICLES FOR SULFIDE SCAVENGING IN MICROBIAL BROTHS**

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### **Background**

Hydrogen sulfide ( $\text{HS}^-$ ) is ubiquitously present in many waste streams originating from industrial activities as well as in sewage. It needs to be removed, as it can be toxic to bacteria, induces concrete corrosion and forms an odour nuisance.  $\text{MnO}_2$  has been proposed for  $\text{HS}^-$  adsorption and oxidation, the addition of such particles to microbial broths may enable selective sulfide scavenging. However potentially negative effects of addition of  $\text{MnO}_2$  microparticles (MPs) on microbial metabolism may occur.

### **Objectives**

Therefore, we evaluated the efficiency of this  $\text{MnO}_2$  MPs based  $\text{HS}^-$  removal strategy and test the effect of the particles and the sulfide scavenging on bacterial growth and microbial community activity.

### **Methods**

Abiotic batch tests were set up to test impact of pH on  $\text{HS}^-$  removal.  $\text{HS}^-$  removal rate after 24h at pH 7.2 was twice that at pH 10.5. Scanning electron microscopy with energy dispersive X-ray spectroscopy showed the presence of sulfur as well as MnS on the particle surface. The effect of  $\text{HS}^-$ ,  $\text{MnO}_2$  MPs and mixtures of both on the growth of single bacterial strains, and mixed microbial community activity were assessed using an optical density microplate reader placed into an anaerobic chamber and biochemical methane potential assays respectively.

### **Conclusions**

The presence of  $\text{MnO}_2$  MPs did not affect bacterial growth under any of the conditions tested. Inhibitory effects due to presence of  $\text{HS}^-$  on bacterial growth were observed, upon addition of  $\text{MnO}_2$  MPs normal growth could be restored. We finally observed that a mixture of  $\text{HS}^-$  and  $\text{MnO}_2$  MPs positively impacted methanogenic activity.



## FEMS-0536

### Pollutant degradation

#### IN STREPTOMYCES, STRONG ANTIBIOTIC PRODUCTION CORRELATES WITH EXTENSIVE STORAGE LIPIDS DEGRADATION AND HIGH ATP GENERATION.

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#### Background

Some *Streptomyces* strains produce antibiotics and others don't. Why? For instance *S. lividans* and *S. coelicolor* are phylogenetically closely related strains possessing the same functional antibiotic biosynthetic pathways but *S. lividans* produces antibiotics at low level whereas *S. coelicolor* produces them at high levels. Interestingly, a dramatic overproduction of antibiotics occurred in *S. lividans* upon interruption of the *ppk* gene. This mutant strain experiences energetic stress (ATP deficit) since Ppk regenerates ATP from ADP and polyphosphate. However, the role of the Ppk in relation to the regulation of antibiotic biosynthesis remains unclear.

#### Objectives

To better understand it we assess the global impact of strong antibiotic production on the cellular metabolism.

#### Methods

To do so proteomic and lipidomic analysis were conducted and the cellular content in polyphosphate, ATP/ADP and free Pi was assayed throughout growth of the three strains of interest grown in phosphate limitation or proficiency.

#### Conclusions

Our results revealed that an active degradation of storage lipids (TriAcylGlycerol, TAG) was taking place in the antibiotic producing strains. TAG degradation generates precursors used directly or indirectly for antibiotics biosynthesis as well as numerous reduced co-factors (FADH<sub>2</sub>) whose re-oxidation by the respiratory chain yields high levels of ATP. Whereas the *ppk* mutant likely mobilizes its storage lipids to re-

establish its energetic balance, the reason of the extensive TAG degradation taking place in *S. coelicolor* is still not understood.

### References

Le Maréchal P *et al.* (2013) Comparative proteomic analysis of the wild-type and the *ppk* mutant of *S. lividans* revealed the importance of storage lipids for antibiotic biosynthesis. AEM 79-19:5907-5917.

**FEMS-2018**  
**Pollutant degradation**

**TOWARDS THE ANAEROBIC-AEROBIC BACTERIAL MINERALIZATION OF CHLORENDIC ACID**

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**Background**

Chlorendic acid (1,4,5,6,7,7-hexachlorobicyclo[2.2.1]-hept-5-ene-2,3-dicarboxylic acid, known as HET acid, or Hetron 92) has been identified in the past as the dead-end product of the biodegradation of the cyclodiene pesticides Aldrin, Endrin, Dieldrin, Endosulfan, Chlordane, and Heptachlor. On the other hand, this compound is still being produced as a flame retardant, and used in respective materials and corrosion-resistant equipment. Chlorendic acid has been considered as non-biodegradable until today.

**Objectives**

Although the hexachlorocyclopentadiene moiety of the compound is highly halogenated and does not carry any hydrogen atom, its dichloroethene substructure may provide the side of bacterial attack by the mechanism of anoxic dehalogenation as known from tetrachloroethylene or polyhalogenated aromatics (halo-respiration), and thus represent the initial side of a biochemical reaction. Further steps of dehalogenation then may proceed aerobically as, for instance, in the catabolic pathway of hexachlorocyclohexane (HCH, Lindane).

**Methods**

We started enrichment experiments with sediments from the River Elbe (Hamburg, Germany) the former contamination of which by pesticides from production plants of chemicals has been well documented, and established a gradient bioreactor system with an anaerobic zone at the bottom towards an aerobic zone at the top. From the latter we could already isolate the dominant bacterium responsible for the mineralization of the (almost) dehalogenated carbon backbone. 16S rDNA analysis revealed this species as 98% identical to *Pseudomonas aeruginosa*.

**Conclusions**

Analyses of the 16S rRNA gene sequences in the anoxic and transition zones will determine the bacterial community structure involved in the catabolic sequence. Identification of intermediates of the combined anaerobic-aerobic pathway is underway.



**FEMS-1628**

**Pollutant degradation**

**MICROBIAL DEGRADATION OF EMERGING TRACE ORGANIC  
CONTAMINANTS: SULFAMETHOXAZOLE, PIRACETAM AND RITALINIC ACID**

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**Background**

Contamination with pharmaceuticals is an emerging environmental problem. Most pharmaceuticals are only partially eliminated in biological wastewater treatment processes, leading to contamination of ground- and surface water. Among them, antibiotics are of particular concern due to potential spread of antibiotic resistance. Sulfamethoxazole (SMX) represents sulfonamides, which belong to most prescribed and consumed antibiotics worldwide. Only a few microbial strains using SMX as a sole carbon source have been isolated and metabolic pathways for most of them have not been described.

The nootropic drugs are a new group of emerging contaminants, very popular these days because of their increasing use as pharmaceuticals and illicit drugs. Some of them, e.g. piracetam and ritalinic acid, have been recently detected in drinking water but so far their potential biodegradation has received very little attention.

**Objectives**

This research aims at isolation and characterization of novel microbial strains capable to degrade SMX, piracetam and ritalinic acid.

**Methods**

Enrichment and isolation of strains was carried out under aerobic conditions using various environmental samples as inocula: wastewater, activated sludge, soil, birds feces etc. Degradation is monitored by HPLC-DAD; new method for ritalinic acid and piracetam have been established; 16S rRNA analysis was performed; radio assays with <sup>14</sup>C labeled ritalinic acid will be carried out.

**Conclusions**

Two novel strains capable of SMX degradation have been isolated. Enrichment cultures with piracetam and ritalinic acid are active and the isolation of strains is ongoing. It is a first attempt to obtain the strains growing on nootropic drugs.

**FEMS-0847**

**Pollutant degradation**

## **DEGRADATION OF TRICLOSAN AND TRICLOCARBAN BY A WASTEWATER MICROORGANISM**

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### **Background**

Since last several decades, biocides like triclosan and triclocarban have been used as a common ingredient in many synthetic products especially in industrial and personal care products. Hence, the widespread use of triclosan and triclocarban has raised major concern over their impact on eco-geological system.

### **Objectives**

We have isolated and identified a triclosan-degrading bacterium from wastewater treatment plant (WWTP) named as *Sphingomonas* sp. strain YL-JM2C.

### **Methods**

Response surface methodology was used to understand the suitable conditions for the degradation of triclosan in strain-YL JM2C. The bacterium can efficiently degrade triclosan (5 mg L<sup>-1</sup>) with biomass between 0.1-0.3 g L<sup>-1</sup> at 30-35 °C with pH 7-8. By GC-MS analysis, the intermediates of triclosan biodegradation were identified as 2,4-dichlorophenol, 2-chlorohydroquinone and catechol. Tracking experiment using <sup>13</sup>C labeled triclosan confirmed that the <sup>13</sup>C labeled triclosan was completely mineralized into carbon dioxide by strain YL-JM2C, and the phospholipid fatty acids (PLFAs) extracted from bacterial cells showed that part of labeled carbon from <sup>13</sup>C labeled triclosan was incorporated into the strain's fatty acids.

### **Conclusions**

These results indicated that the bacterium was able to utilize triclosan as a carbon source. We also observed that the bacterium was also able to degrade 30 to 35% of triclocarban (4 mg L<sup>-1</sup>). In *Sphingomonas* sp. strain YL-JM2C, triclocarban was initially hydrolyzed into 3,4-dichloroaniline and 4-chloroaniline. 3,4-Dichloroaniline was further transformed to 4-chlorocatechol via 4-chloroaniline. As per our knowledge, this is the first report of bacterial degradation of triclocarban by a pure isolated bacterial strain.

**FEMS-2571**

**Secretion**

**COMPARATIVE AND TRANSCRIPTIONAL ANALYSIS OF THE PREDICTED SECRETOME IN THE LIGNOCELLULOSE DEGRADING BASIDIOMYCETE PLEUROTUS OSTREATUS**

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**Background**

Fungi interact with their environment by means of secreted proteins to obtain nutrients, elicit responses and modify their surroundings. Consequently, lifestyle influences the set of fungal secreted proteins.

**Objectives**

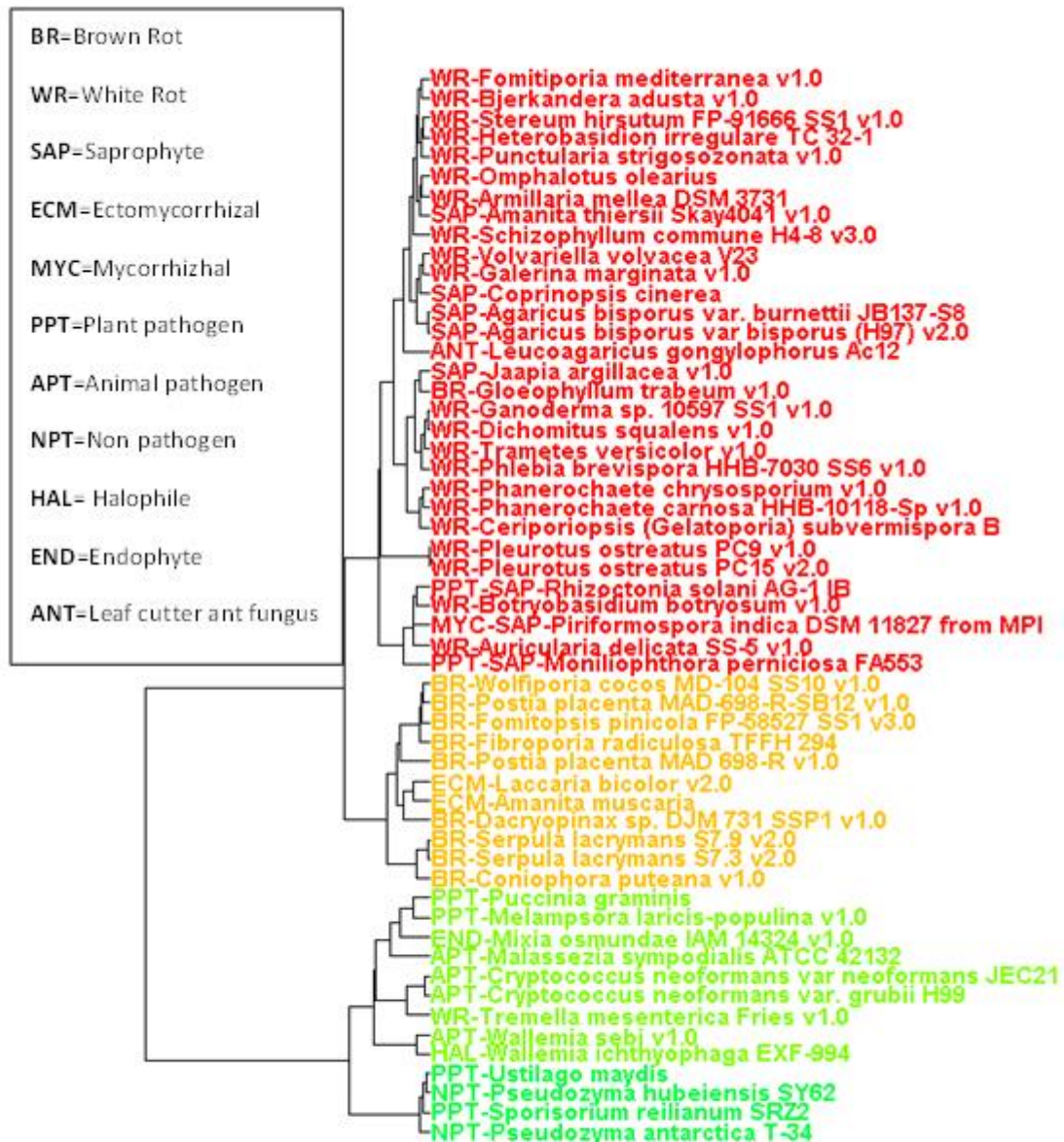
To perform a combined bioinformatics-transcriptomics study of the secretome of the basidiomycete *Pleurotus ostreatus* and to explore the conservation of these secreted proteins across the Basidiomycota phylum

**Methods**

We identified bioinformatically the set of secretable proteins in two monokaryotic strains (haplotypes) of the white-rot basidiomycete *P. ostreatus* (PC9 and PC15) using the web pipeline SECRETOOL [1]. Then, we performed two RNA-seq analyses to study the relationship between the functional profile of the predicted secretome and the expression level of each group. Finally, we used the set of proteins secreted by *P. ostreatus* as a query to search for similar proteins in the fungal genomes released in JGI Mycocosm[2]

**Conclusions**





**Figure 1:** Dendrogram showing several basidiomycetes arranged by presence of similar proteins (E-value<e-20) to PC15 predicted to be secreted proteins

538 and 554 protein models were predicted to be secreted (4.41% and 4.77% of PC9 and PC15 gene models, respectively). The functional annotation of these proteins revealed the unknown (37.2%), glycosyl hydrolases (26.5%) and red-ox enzymes (11.54%) as the main functional groups, in a similar distribution for the two strains. The expression level of these groups further enhances the relevance of the unknown group and was significantly different in the two strains (revealing different responses to the same environment). Furthermore, the presence of similar proteins to *P. ostreatus* secreted proteins in other basidiomycetes was used to cluster them into

groups coherent with their particular lifestyles rather than with their corresponding phylogenetic positions.

**FEMS-0827**

**Secretion**

## **NEW INSIGHTS INTO THE T6SS OF PSEUDOMONAS AERUGINOSA**

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### **Background**

*Pseudomonas aeruginosa* is a gram-negative opportunistic pathogen capable of living in a wide variety of environmental niches and is the third most common cause of all hospital-acquired infections. *P. aeruginosa* utilises a range of secretion systems and virulence factors to enable it to prosper. The Type VI Secretion System (T6SS) is one such factor. This nanomachine can modulate invasion of epithelial cells and is widely known for its ability to facilitate the delivery of effector proteins into both Eukaryotic cells and Bacterial competitors.

### **Objectives**

The majority of *P. aeruginosa* strains have three complete T6SS clusters [H1-, H2-, and H3-T6SS] however additional 'orphan' genes or short operons are present in the *P. aeruginosa* genomes, which have the characteristics of T6SS components. In this study we have employed a range of molecular microbial techniques to further investigate components of the T6SS and related systems.

### **Methods**

Through the construction of *lacZ* fusions, transposon mutagenesis, specific gene deletions, complementation and labelling with V5 tags we have identified new secreted products and mechanistic insights that contribute to the function of the *P. aeruginosa* T6SS. We also characterized global regulatory networks that are instrumental to the expression of genes encoding either core components of the T6SS machine or cognate T6SS effectors.

### **Conclusions**

Overall secretion systems are of vital importance for Bacteria to interact with their environment and cause infection. A detailed knowledge of the mechanisms of secretion combined with an understanding of the role of the secreted factors should provide the basis for the development of new therapies to combat bacterial infections.



**FEMS-2581**

**Secretion**

## **EXPRESSION OF THE MENINGOCOCCAL AUTOTRANSPORTER AUTB AND ITS IMPACT ON BIOFILM FORMATION**

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### **Background**

Autotransporters are secreted proteins of Gram-negative bacteria often involved in virulence. They consist of a translocator domain that inserts as a  $\beta$ -barrel in the outer membrane and a fused passenger domain that is translocated across the outer membrane. Genomes of the human pathogen *Neisseria meningitidis* contain eight autotransporter genes. Seven have been characterized to a certain extent, but the autotransporter AutB remains poorly studied. Previous studies suggested that the *autB* gene is a pseudogene.

### **Objectives**

Our objective was to analyze whether AutB can be expressed and to elucidate its possible function.

### **Methods**

In this study we used a combination of genomic and proteomic assays to determinate the expression of the autotransporter. Included were also diverse functional assays to analyse its function.

### **Conclusions**

Bioinformatics analysis of available genome sequences indicated that AutB expression is prone to phase variation, and can be inhibited by the presence of premature stop codons or other genetic disruptions. However, several genome sequences contain an intact *autB* gene. Western blotting and RT-PCR assays demonstrated the expression of AutB in strains with an intact gene. Proteinase K-accessibility assays evidenced that AutB is secreted but its passenger remains attached to the cell surface. Functional assays revealed the involvement of AutB in biofilm formation in a strain-dependent mode. We conclude that *autB* is a pseudogene only in some strains and that AutB may have a relevant function in the formation of bacterial communities.

**FEMS-2873**

**Secretion**

**THE OLOGOMERIZATION STATE OF THE TYPE III EFFECTOR/CHAPERONE COMPLEX CAN PROMOTE ITS RECOGNITION BY THE TYPE III SECRETION SYSTEM IN SALMONELLA.**

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**Background**

Many bacterial pathogens use Type Three Secretion System (T3SS) to inject virulence factors, named effectors, directly into the cytoplasm of target eukaryotic cells. Before being translocated, effectors are maintained in a secretion-competent state by interacting with specific chaperones. The molecular mechanism of the recognition process between the effector/chaperone complex and the T3SS is still unclear but a highly conserved hexameric-ring-shaped ATPase localized at the basis of the needle complex has a critical role in the specific recognition process of chaperone-effector complex.

**Objectives**

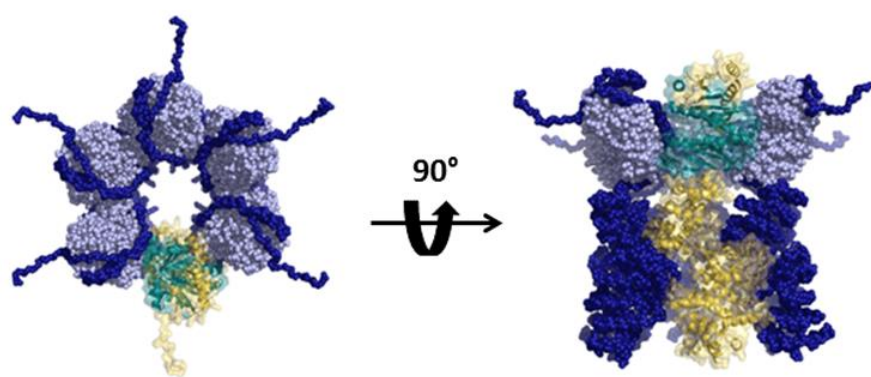
As previous works showed that chaperones and effectors share structural motifs that could be at the origin of the recognition by TTSS, we investigated the structure of the complex between SopB, and its cognate chaperone SigE that is the form recognized by TTSS.

**Methods**

In this work we performed a biochemical and structural characterization of *Salmonella* SopB/SigE chaperone/effector complex by SAXS.

**Conclusions**

Our results showed that the SopB/SigE complex is assembled in dynamic homo-hexameric-ring-shaped structures with an internal tunnel. In this ring, the chaperone maintains a disordered N-terminal end of SopB molecules, in good position to be reached and processed by the T3SS. This ring dimensionally fits the ring-organized molecules of the injectisome including ATPase hexameric ring; this organization suggests that this structural feature is important for the ATPase recognition by T3SS. As effectors share neither sequence nor structural identity, the quaternary oligomeric structure could constitute a strategy evolved to promote the specificity and efficiency of T3SS recognition.



**FEMS-2658**

**Secretion**

**DECIPHERING THE MOLECULAR STRUCTURE AND FUNCTION OF VIRB4-LIKE ATPASES IN NUCLEOPROTEIN IMPORT AND EXPORT**

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**Background**

VirB4-like proteins are associated with all bacterial Type 4 Secretion Systems (T4SS) described to date. These signature ATPases function in assembly of the T4SS channel and biogenesis of extracellular pili. They are also required for translocation of secretion substrates and nucleoprotein uptake during pilus-mediated phage infection.

**Objectives**

Very little is known about the regulation of VirB4-like ATPases in protein trafficking. Our paradigm to study control of VirB4-like ATPases is TraC of the F-like conjugative plasmid R1. Here we test the hypotheses that ATPase activity is controlled by (i) oligomeric state and (ii) intra-molecularly, by auto-inhibition.

**Methods**

Full length and truncated alleles of traC were modified with a strep-tag and evaluated functionally by complementation of conjugative transfer of a  $\Delta traC$  derivative of R1. Multimer formation, nucleotide binding and hydrolysis by purified TraC proteins were measured under various conditions in the presence and absence of protein interaction partners using NMR and biochemical techniques.

**Conclusions**

Full length TraC behaves as a monomer in solution with and without a 20-fold molar excess of non-hydrolysable ATP. This form binds but does not hydrolyze nucleotide. Preliminary data indicates that proteolytic processing of the protein stimulates both the formation of apparent hexamers and enzyme activity.

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## **FEMS-1927**

### **Secretion**

#### **MOLECULAR INVESTIGATION OF THE BASEPLATE ASSEMBLY OF THE TYPE VI SECRETION SYSTEM OF PSEUDOMONAS AERUGINOSA**

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### **Background**

The type VI secretion system (T6SS) involves ~13 core proteins and shares similarities with the tail of the bacteriophage T4. The current model of the structural organization of the T6SS suggests that its core components are assembled in several subcomplexes that are associated to form a dynamic phage-like machinery. A first set of T6SS components forms a trans-envelope complex which consists of the two inner membrane proteins TssM and TssL, and the outer membrane lipoprotein TssJ. A second group of T6SS proteins forms the bacteriophage tail-like structure. It includes the components Hcp and VgrG, and the sheath-like structure made of the two proteins TssB and TssC. Hcp proteins are hexameric rings which are able to form a rigid tube capped by the puncturing device made by VgrG proteins. Contraction of the TssB/TssC sheath propels the Hcp-VgrG structure, which ultimately results in the injection of toxins into target cells.

### **Objectives**

The remaining T6SS core components are likely to form what is known as the baseplate structure in the phage. In the T6SS, it connects the tail-like structure to the membrane subcomplex. Functional and structural information about these T6SS proteins as well as their assembly within the machinery remain poorly described.

### **Methods**

Here, we used biochemical and electron microscopy techniques to determine the structure and function of individual components of the T6SS in *Pseudomonas aeruginosa*. Additionally, using a systematic analysis of protein-protein interaction, we determined the interaction network between T6SS proteins.

## **Conclusions**

Overall these data led to propose a comprehensive working model of the T6SS.

**FEMS-1550**

**Secretion**

**TYPE VI SECRETION SYSTEM AS A VIRULENCE MECHANISM OF MAMMARY PATHOGENIC ESCHERICHIA COLI**

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**Background**

Mastitis, infection and inflammation of the mammary gland, is a well-known problem in the dairy industry, affecting cows worldwide and causing considerable financial losses. Multiple bacterial species can cause mastitis and *E. coli* is often involved. Despite many years of mastitis research, no efficient measures exist to prevent or treat the disease, and only little is known about specific virulence factors of the bacteria.

**Objectives**

Our goal is to understand the molecular mechanisms of host-pathogen interactions in the mammary gland and relate them to disease processes, in hope that understanding these mechanisms will lead to development of novel tools to combat *E. coli* mastitis.

**Methods**

Using genome sequencing and analysis of six clinical isolates, we found that type VI secretion system (T6SS) gene clusters are present in all. Furthermore, using unbiased screening of strains for reduced colonization, fitness and virulence in our murine mastitis model, we have identified in P4-NR strain a new pathogenicity island encoding the core components of T6SS and its hallmark effectors Hcp, VgrG and Rhs. Next, we have shown that specific deletions of T6SS genes reduced *in vivo* pathogenicity.

**Conclusions**

Based on our results we hypothesize that T6SS is an important virulence mechanism of MPEC. To our knowledge, we are the first to describe relevance of T6SS in the pathogenesis of mastitis. We intend to validate our findings in dairy cows and field strains and study the molecular mechanisms of T6SS associated with MPEC virulence. The identified mechanism may provide new targets for diagnostic, preventive and therapeutic intervention.

## **FEMS-1974**

### **Secretion**

#### **BACTERIAL KILLING VIA A TYPE IV SECRETION SYSTEM**

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### **Background**

Type IV secretion systems (T4SSs) are multiprotein complexes that transport effector proteins and protein-DNA complexes through bacterial membranes to the extracellular milieu or directly into the cytoplasm of other cells. They are essential for host colonization by many medically important microbes as well as for horizontal transfer of genetic material between bacteria and from bacteria to plants. Many bacteria of the family Xanthomonadaceae, that occupy diverse environmental niches, carry a T4SS with unknown function but with several characteristics that distinguishes it from other T4SSs.

### **Objectives**

The aim of this study is to determine the function of the Xanthomonadaceae T4SS.

### **Methods**

We have used structural biology, spectroscopy, enzymology and novel bacterial competition and secretion experiments to address this question.

### **Conclusions**

The *Xanthomonas citri* (Xac) T4SS provides these cells the capacity to kill other gram-negative bacterial species in a contact-dependent manner. The secretion of one Type IV bacterial effector protein is shown to require a conserved C-terminal domain and its bacteriolytic activity is neutralized by a cognate immunity protein whose 3D structure is similar to peptidoglycan hydrolase inhibitors. This is the first demonstration of the involvement of a T4SS in bacterial killing and points to this special class of T4SS as a mediator of both antagonistic and cooperative interbacterial interactions and therefore an important driving force in the evolution of bacterial species.



**FEMS-2957**

**Secretion**

## **STUDIES ON THE REGULATION OF TYPE VI SECRETION SYSTEMS IN ESCHERICHIA COLI**

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### **Background**

Type VI secretion systems (T6SS) are complex protein transport machineries and widespread among pathogenic Gram-negative bacteria. Accordingly, one could assume that they may contribute directly or indirectly to pathogenesis; for example, by targeting eukaryotic host cells or by competing with other bacteria in microbial populations.

### **Objectives**

The genomes of the uropathogenic *Escherichia coli* (UPEC) strains often harbor one or more genomic islands encoding putative T6SSs. As the expression of T6SSs seems to be tightly regulated, most of the secretion systems are inactive under standard laboratory conditions. The screening for potential regulators directing gene expression of T6SSs is a first step to get a better understanding under which conditions these secretion systems may be active and contribute to the fitness and/or pathogenicity of *E. coli*.

### **Methods**

To elucidate the transcriptional regulation of the T6SSs in UPEC strains, chromosomal and plasmid-based promoter-reporter gene fusions to core genes of both genomic islands have been constructed. The promoter activity has been tested in different *E. coli* K-12 deletion backgrounds as well as in various wild type *E. coli* strains.

### **Conclusions**

The screening using the promoter-reporter gene fusion demonstrated that the availability of nucleoid-associated proteins and the growth phase, i.e. the stationary phase, affect transcription of genes coding for core elements of T6SSs.

**FEMS-0660**

**Secretion**

**TOWARDS A BETTER UNDERSTANDING OF THE BACTERIAL TYPE II  
SECRETION PATHWAY**

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S. BLEVES<sup>1</sup>, C. SOSCIA<sup>1</sup>, M. TEGON<sup>2</sup>, C. CAMBILLAU<sup>2</sup>, K. FOREST<sup>3</sup>,  
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**Background**

The bacterial type II secretion system (T2SS) is unique in its ability to promote the transport of large folded and sometimes multimeric proteins. In this secretion process, exoproteins are first translocated into the periplasm. The final release into the medium requires a multiprotein complex called the secreton.

**Objectives**

Although the 12 individual components of the secreton have been identified, its mode of action remains obscure.

**Methods**

We set up various dedicated *in vitro* and *in vivo* protein-protein interaction experiments to identify the *Pseudomonas aeruginosa* Xcp T2SS periplasmic interactome.

**Conclusions**

BIAcore experiments revealed that three Xcp components, XcpP, the secretin XcpQ, and the pseudopilus tip, directly and specifically interact with secreted exoproteins. Affinity chromatography co-purification indicated that the XcpY periplasmic domain interacts with the secreted substrate and a component of the pseudopilus tip XcpW. Interestingly, the periplasmic domain of another member of the Xcp inner membrane platform, XcpZ co-elutes with the XcpY/substrate and the XcpY/XcpW complexes during affinity chromatography. Finally the direct interaction between the secreted substrate and XcpY was confirmed by *in situ* photo-crosslinking. All together, our results allowed us to propose the most advanced integrative model of Xcp T2SS assembly and function.

**FEMS-2044**

**Secretion**

## **BACTERIAL TYPE III SECRETION NEEDLE COMPLEX: A STOICHIOMETRIC ANALYSIS**

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### **Background**

Bacterial type III secretion systems are cell-envelope spanning effector protein-delivery machines employed by Gram negative pathogens and symbionts. The membrane-embedded core unit of these secretion systems is termed needle complex. It can be divided into a base that anchors the complex to the inner and outer membranes, a hollow filament composed of inner rod and needle subunits that serves as conduit for substrate proteins, and an export apparatus facilitating substrate translocation located at the center of the base.

### **Objectives**

While the low resolution structural analysis of the base has revealed the stoichiometry of this large ring-forming complex composed of three different proteins, the stoichiometries of the five hydrophobic export apparatus components and of the inner rod are largely unknown.

### **Methods**

We employed a mass-spectrometry based peptide-concatenated standard strategy to evaluate the stoichiometry of the entire needle complex.

### **Conclusions**

Using this strategy, we could validate the hitherto suggested stoichiometries of the base components and major export apparatus protein. We further propose a stoichiometry of 5:1:1:1 for the minor export apparatus components SpaP, SpaQ, and SpaR and for the switch-protein SpaS of the type III secretion system located on pathogenicity island 1 of *Salmonella* Typhimurium. The herein presented data also suggest that the inner rod is merely a rod but rather a disc because of the low stoichiometry observed.

Even though the complexity and hydrophobic character of the evaluated components allowed only the obtainment of approximate stoichiometries in this study, the gained results provide the first complete picture and a valuable framework for further investigations.



**FEMS-1267**

**Toxin / antitoxin: activities and functions**

**CHARACTERIZATION OF A NEWLY DISCOVERED TOXIN-ANTITOXIN FAMILY  
PREDICTED TO HAVE AN ADP-RIBOSYLATING TOXIN**

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**Background**

Toxin-antitoxin (TA) systems are highly abundant in bacteria and are typically composed of adjacently linked genes that encode both stable toxin and its unstable 'antidote'. TA systems were shown to have a diverse set of physiological roles that range from phage defense to bacterial persistence. We recently reported the discovery of six new types of TA systems (Sberro *et al.* Discovery of functional toxin/antitoxin systems in bacteria by shotgun cloning. Mol Cell. 2013 50(1):136-48).

**Objectives**

Here, we aimed in characterizing one of these newly discovered TA systems, Pmen TA. We propose that this toxin is involved in ADP-ribosylation, a post-transcriptional modification of crucial components within the bacterial cell. This mechanism is observed in a variety of bacterial exotoxins.

**Methods**

We use a combination of bioinformatics tools, molecular biology and biochemistry in order to attain initial characterization of this TA system after expressing it in *E.coli*.

**Conclusions**

The Pmen Toxin is predicted to have an NAD-binding domain that allows ADP-ribosylation activity. The anti-toxin is unstable and physically binds the toxin. It has an N-terminal domain, known to bind ADP-ribose, and its removal eliminates the rescue effect against the toxin. The conserved residues of the toxin resemble the core active site residues in other ADP-ribosylating toxins, such as Cholera toxin. Substitution of the most conserved active site residue leads to abolishment of toxicity. Possible common evolutionary origin between toxin-antitoxin systems and lethal exotoxins suggests that TA systems may be a genetic source for the emergence of pathogen-related toxins.

**FEMS-2485**

**Toxin / antitoxin: activities and functions**

**THE CELLULAR LOCALIZATION AND RIBOSOME ASSOCIATION OF ACINETOBACTER BAUMANNII TYPE II TOXIN-ANTITOXIN SYSTEM COMPONENTS**

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**Background**

Most prokaryotic genomes, including human pathogens, code for toxin-antitoxin (TA) systems, usually consisting of a toxin and its cognate antitoxin, which counteracts toxin activity. TA systems have been associated with a variety of biological functions, including general stress response, biofilm formation and pathogenicity. *Acinetobacter baumannii* is multidrug-resistant pathogen causing serious hospital-acquired infections worldwide. The internally encoded TA systems can be a useful tool in understanding and preventing the spread of multi-drug resistant *A. baumannii*.

**Objectives**

Recently, at least five functional *A. baumannii* TA systems (RelBE, HicAB, HigBA, SplTA, CheTA) were discovered. Their toxins were shown to inhibit translation in *Escherichia coli* cells. In order to characterize the *A. baumannii* toxins in more detail, we have investigated their cellular localization and ribosome-association.

**Methods**

Series of inducible *E. coli* expression vectors encoding *A. baumannii* TA components, fused with fluorescent proteins have been constructed. The functionality of fusion proteins in *E. coli* was confirmed using the kill-rescue assay. The ability of toxins to interact with ribosome was tested using ribosome fractionation, cellular localization was accessed by fluorescence microscopy.

**Conclusions**

Microscopy of *E. coli* where the fusion toxins, alone or with their cognate antitoxins, were expressed, has shown either polar or whole cell localization pattern. The analysis of fluorescence profiles of *E. coli* ribosomal fractions has demonstrated only RelE associated with ribosome, while association with the ribosome is unlikely for HicA, HigB and SpIT toxins of *A. baumannii*. The latter toxins might cleave RNA independently from ribosome, or inhibit translation by other mechanisms.

**FEMS-0573**

**Toxin / antitoxin: activities and functions**

## **ALLELOPATHIC INTERACTIONS BETWEEN MICROCYSTIS AERUGINOSA 7806 AND DESMODESMUS SUBSPICATUS IN A CO-CULTURE EXPERIMENTAL MODEL**

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### **Background**

Microcystins, produced primarily by cyanobacterium *Microcystis aeruginosa*, are the most commonly studied cyanotoxins (1). Most studies have focused only on the toxic effects of microcystins on humans and animals while their physiological or ecological roles have not been elucidated. Therefore, there is a considerable interest in understanding the importance of cyanotoxins in natural environments (2).

### **Objectives**

The aim of the present study was to clarify the ecological significance of microcystins in algal succession.

### **Methods**

*Desmodesmus subspicatus* was cultured in a medium together with dialysis tubing containing *M. aeruginosa* 7806. Cultures were incubated under  $30 \mu\text{Em}^{-2}\text{s}^{-1}$  continuous irradiation at 23°C for 4 weeks. Microcystin-LR was quantified using mass spectrophotometer and algal density was obtained by measuring the OD<sub>750</sub>.

### **Conclusions**

Microcystin-LR was determined in the media after two weeks but there was no change in *D. subspicatus* growth rate. After 4 weeks incubation, *D. subspicatus* growth decreased. Using co-cultivation method gave the opportunity to investigate the algal interactions at continuous toxin production. By continuing the growth of *M. aeruginosa*, toxin concentration increased and caused a decrease in *D. subspicatus* growth. Results provided evidences to suppose the role of toxin as an allelochemical in algal interactions.

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**FEMS-2621**

**Toxin / antitoxin: activities and functions**

**FIC PROTEIN OF THE NICHE –ADAPTED PATHOGEN CAMPYLOBACTER FETUS SUBSP. VENEREALIS FORM CIS- AND TRANS-ACTING TOXIN-ANTITOXIN SYSTEMS.**

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**Background**

*Campylobacter fetus* cause human infection and are important pathogens. Recent comparative genomics of *C. fetus* subspecies revealed *Fic* loci encoding Fido superfamily proteins, which may contribute to niche adaptation and pathogenicity.

**Objectives**

We analysed function of 4 *Fic* loci organized as toxin-antitoxin (TA) modules on the chromosome and ICE of *C. fetus* subsp. *Venerialis* 84-112.

**Methods**

The *C. fetus* proteins and mutant variants were expressed ectopically in *E. coli*, yeast and in transiently transfected HeLa cells. Prevalence of *fic* genes in 102 *C. fetus* isolates was surveyed with PCR

**Conclusions**

We show that *Fic* proteins are cytotoxic to human cells but not *S. cerevisiae*. The *fic* loci are organized as TA modules on the chromosome and ICE of *C. fetus* subsp. *venerialis* 84-112. Expression in *E. coli* validated the cytotoxic and neutralizing activities of the proteins, providing the first functional evidence for TA systems in *Campylobacter*. Reversal of *fic*-mediated filamentation and growth inhibition in *E. coli* also revealed antitoxin crossreactivity between loci. Key active site residues involved in adenylation by *Fic* proteins are conserved in *Fic1*, *Fic3* and *Fic4*, but degenerated in *Fic2*. We show the non-canonical *Fic2* disrupts assembly of *E. coli* ribosomes. *fic* genes are prevalent in *C. fetus* subsp. *venerialis* but not generally conserved among *Campylobacters*. Strikingly, homologous genes are found in some *Campylobacters* and unrelated pathogens adapted to the human and animal

urogenital tract. *C. fetus* *fic* genes thus appear to be important to adaptation and virulence in this niche.

**FEMS-1515**

**Toxin / antitoxin: activities and functions**

## **TOXIN-ANTITOXIN SYSTEMS ON ANTIBIOTIC RESISTANCE PLASMIDS: JUST PLASMID MAINTENANCE?**

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### **Background**

Toxin-Antitoxin (TA) systems are small genetic elements composed of a toxin gene and its cognate antitoxin. They were originally discovered as plasmid-borne loci that promote plasmid maintenance by killing daughter cells that have lost their TA encoding plasmid, a phenomenon known as post-segregational killing. Nowadays it is known that a great diversity of plasmids, including the antibiotic resistance (AbR) plasmids, encodes one or even more TA systems. The widespread dissemination of antibiotic resistance genes it is ascribed to the spread of bacterial plasmids encoding these genes and this successful dissemination it is attributed in part to TA systems and its participation in plasmid maintenance. However, there are some lights indicating that plasmidial TA systems could participate in functions other than plasmid maintenance.

### **Objectives**

Our main aim was to elucidate the diversity and function of TA systems encoded on AbR plasmids. Specifically to characterize a new putative TA system encoded on an IncX4 plasmid.

### **Methods**

By bioinformatic tools a novel putative TA system was identified in pJIE143, an IncX4 plasmid carrying *bla*<sub>CTX-M-15</sub> from an *Escherichia coli* ST131 isolate. We analyzed the effect of the expression of this system on *E. coli* growth and its participation on plasmid maintenance.

### **Conclusions**

pJIE143 encodes a novel TA system that appears to be related exclusively to IncX plasmids. The characteristics and putative functions of this novel TA system will be discussed in this work. These results reveal that AbR plasmids could contain an underappreciated diversity of TA systems, which could have functions further of plasmid maintenance.





**FEMS-1272**

**Toxin / antitoxin: activities and functions**

**TOXICITY OF TWO ENTOMOPATHOGENIC FUNGI, BEAUVERIA BASSIANA AND LECANICILLIUM MUSCARIUM AGAINST A FIELD COLLECTED STRAIN OF GERMAN COCKROACH BLATTELLA GERMANICA FROM HOSPITALS IN SANANDAJ, IRAN**

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**Background**

The German cockroach, *Blattella germanica* (L.) has been recognized as a serious health problem throughout the world. Control failures due to insecticide resistance and chemical contamination of environment have led some researchers focus on the other alternative strategy controls. Microbial insecticides such as those containing entomopathogenic fungi could be of high significance. *Lecanicillium muscarium* and *Beauveria bassiana* grow naturally in soils throughout the world and act as a parasite on various arthropod species, causing white muscardine disease. Thus, these two species could be considered as entomopathogenic fungi.

**Objectives**

The current study conducted to evaluate the toxicity of *Beauveria bassiana* and *Lecanicillium muscarium* against German cockroach, *Blattella germanica*.

**Methods**

Conidial formulations of *L. muscarium* (PTCC 5184) and *B. bassiana* (PTCC5197) were prepared in aqueous suspensions with Tween 20. Bioassays were performed using two methods including submersion of cockroaches in conidial suspension and baiting. Data were analyzed by Probit program and LC50 and LC90 were estimated.

**Conclusions**

The obtained results indicated that both fungi species were toxic against German cockroach however; *Beauveria bassiana* was significantly 4.8 fold more toxic than *L. muscarium* against German cockroach using submersion method.



**FEMS-2091**

**Toxin / antitoxin: activities and functions**

## **REGULATION OF TOXIN-ANTITOXIN SYSTEMS WITHIN THE SOS REGULON IN E. COLI**

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### **Background**

TA systems (TAs) encode a stable toxic protein and its cognate unstable antitoxin that is either an antisense RNA (type I) or a protein (type II). Under steady-state conditions, both components are produced and cells are growing normally. Under conditions in which the antitoxin:toxin ratio is perturbed in favor of toxin, cell growth can be impaired. Therefore, maintaining an appropriate ratio is essential for viability. While negative autoregulation is observed for type II systems, few information is available for type I. Some of these TAs (*symER*, *tisAB-istR* and *dinQ-agr*) are part of the SOS regulon, as well as two type II TAs (*dinJ-yafQ* and *yafNO*).

### **Objectives**

Our objective is to investigate the transcriptional regulation of these TAs at the population and single cell levels under steady-state growth and SOS conditions.

### **Methods**

Approaches based on fluorescent reporters fused to TA promoters combined with flow cytometry and microscopy analysis are currently being developed.

### **Conclusions**

Our data confirmed that the *dinJ-yafQ* and *symE* promoters are activated during SOS response, although at different timing and levels. Interestingly, the *dinJ-yafQ* promoter is active both in wild-type and *dinJ-yafQ* mutant strains, indicating that SOS regulation prevails on autoregulation. Furthermore, heterogeneity in promoter activity was observed at the single cell level, suggesting that phenotypic heterogeneity might play a role in the SOS response. This will be further investigated.

**FEMS-2327**

**Toxin / antitoxin: activities and functions**

## **EVOLUTIONARY RELATIONSHIPS BETWEEN TYPE II TOXIN-ANTITOXIN SYSTEMS AND POLYMORPHIC TOXIN SYSTEMS**

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### **Background**

Type II toxin-antitoxin systems (TAs) are small bicistronic modules encoding a toxic protein and its cognate antitoxin protein. TAs are widespread in bacterial and archeal genomes.

### **Objectives**

Our hypothesis is that TAs might constitute a reservoir of toxic domains that are recruited by polymorphic toxin systems.

### **Methods**

Polymorphic toxin systems, such as CDI and Rhs, are composed of large conserved proteins containing variable carboxy-terminal (CdiA-CT or RhsA-CT) domains showing toxic activity and small immunity proteins. Genetic organization of these systems is analogous to that of TAs: a toxic gene or domain followed by an antitoxin or immunity gene. A bioinformatics approach is currently developed to evaluate whether CdiA-CT and RhsA-CT toxic domains are found in TAs. Domains are collected and HMM profiles are built. Bacterial genomes are scrutinized for the presence of these domains under TA organization. Functionality of these systems is tested in *E. coli*. Toxin activity and targets are then investigated.

### **Conclusions**

We identified and experimentally validated novel toxic domains that are found in TAs organization and in CDI and/or Rhs systems. Upon overexpression, toxins from TAs and CdiA-CT/RhsA-CT domains are toxic and antagonized by the expression of cognate antitoxin and/or immunity proteins. These domains were shown to inhibit translation. These data indicate that toxic domains are indeed shared by TAs and polymorphic toxin systems. Recruitment of toxic domains encoded by TAs by more

sophisticated systems might constitute a strong selective pressure on TAs and might explain why these small modules are evolutionary successful.

**FEMS-0753**

**Toxin / antitoxin: activities and functions**

**NOVEL FAMILY OF TOXINS WITH ACETYLTRANSFERASE DOMAIN  
CONSTITUTE TYPE II TOXIN-ANTITOXIN SYSTEMS**

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**Background**

Type II toxin-antitoxin (TA) systems are small genetic loci coding for a toxic protein and its antidote. Such systems are widespread in bacterial and archaeal genomes. These systems stabilize mobile genetic elements and may act as stress response elements. Toxins of type II TA systems are most commonly mRNAses, with the exception of gyrase inhibitors and several families that have more elaborate enzymatic functions. We have found novel class of toxins that possess acetyltransferase domain and constitute typical type II TA systems.

**Objectives**

To functionally describe TA modules of this novel family and explore mechanism of toxicity of acetyltransferase domain toxin.

**Methods**

TA systems from *Escherichia coli* and *Vibrio cholerae* have been functionally validated by killing/rescue assay. Activity of toxin has been tested by measuring rates of replication, transcription and translation, mRNAse activity by Northern blots analysis. Mutational analysis on acetyltransferase active site has been performed. TA complex has been purified and separated by affinity chromatography. Bioinformatics analysis has been performed to describe the prevalence and diversity of this novel family.

**Conclusions**

Small acetyltransferase domain proteins found in pair with RHH-domain antitoxins constitute functional TA pairs. When expressed together they form a heteromultimeric complex of 2 toxins and 2 antitoxins. When expressed alone toxins possessing acetyltransferase domain inhibit translation without degrading mRNAs and therefore exhibit novel toxicity mechanism. Mutations in the acetyltransferase active site abolish toxicity, strongly indicating that toxicity relies on target(s) acetylation. Detailed analysis of translation inhibition *in vitro* is currently under progress and should reveal the target(s) of the toxin.

**FEMS-2218**

**Toxin / antitoxin: activities and functions**

**CLAMP ALLOSTERY AND STEREOSELECTIVITY GOVERN THE MOLECULAR MECHANISM OF PROTEIN TRANSLOCATION THROUGH THE ANTHRAX TOXIN PROTECTIVE ANTIGEN CHANNEL**

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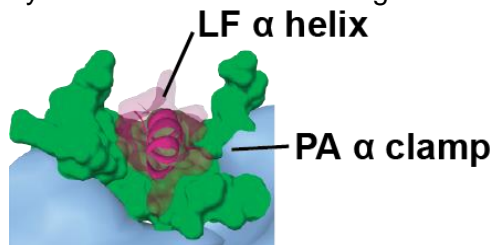
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**Background**

Anthrax toxin, composed of protective antigen (PA) and lethal (LF) and edema (EF) factors, is a model system for transmembrane protein translocation. The PA component self-assembles into a proton motive force (PMF)-driven channel, which translocates LF and EF into target cells. The channel contains multiple nonspecific polypeptide-clamp sites, which catalyze unfolding and translocation. It is unknown how clamps facilitate transport while avoiding tightly bound kinetic traps.

**Objectives**

As the  $\alpha$  clamp, specifically recognizes  $\alpha$  helix, we asked how the conformational dynamics of the translocating chain might avoid kinetic traps during translocation.



**Methods**

~50-residue peptides (based upon the sequence of LF) were synthesized with identical sequences but variable stereochemistries to control for side-chain chemistry while manipulating helical sense and backbone flexibility. Single-channel and ensemble planar lipid bilayer electrophysiology monitored peptide binding, dissociation, and translocation.

**Conclusions**

Cooperative binding and allosteric modulation of translocation were only observed with an isotactic peptide synthesized only with L-enantiomers ( $\alpha$ L). Cooperativity depended on acidic pH and the channel's  $\alpha$ -clamp and  $\phi$ -clamp sites. Incorporation of D-enantiomers disrupted cooperativity, allosteric control of translocation, and slowed the rate of translocation. The translocation of  $\alpha$ L occurs via a subsecond series of consecutive irreversible transitions and is unlike the translocation of a syndiotactic

peptide, synthesized with alternating L- and D-enantiomers ( $\alpha$ LD), which translocates 100-times slower (as slow as 776-residue LF). Therefore, to avoid kinetic traps, which may otherwise impede translocation, the channel's clamps operate allosterically through efficient, repeated, stereospecific contact along the length of the translocating peptide chain.



**FEMS-2665**

**Toxin / antitoxin: activities and functions**

**TWO VAPBC-TYPE TOXIN-ANTITOXIN MODULES OF SINORHIZOBIUM MELILOTI INFLUENCE SYMBIOTIC EFFICIENCY AND NODULE SENESCENCE OF MEDICAGO SP.**

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**Background**

The symbiotic interaction between the bacterium *Sinorhizobium meliloti* and the legume *Medicago sp* results in the development of new root organs, the nodules, where the differentiated bacteria (the bacteroids) reduce atmospheric N<sub>2</sub> to ammonia. A few weeks after symbiosis establishment, nodules present a premature senescence.

**Objectives**

Since VapBC-type Toxin-Antitoxin (TA) systems of pathogenic bacteria is involved in the survival of the bacteria, we examined the role of *S. meliloti* VapBC modules in bacteroid viability and nodule senescence. The *vapBC* genes form an operon negatively regulated by the TA complex. Free toxin (VapC) has an RNase activity that is neutralized by the antitoxin (VapB).

**Methods**

We showed that, during the symbiotic interaction, a mutation in the *vapC5* toxin of the VapBC5 module, leads to a higher nitrogen-fixing activity, plant yield increase and a delayed nodule senescent phenotype. Thus, inactivation of this toxin improves symbiotic efficiency (Lipuma et al., 2014). We obtained an opposite phenotype with another mutant of toxin deletion (*vapC7*) : aberrant nodules, where nitrogen fixation is nearly abolished. Nodule ultrastructure analysis and flow cytometry demonstrated that bacteroid differentiation was followed by a rapid bacterial death leading to early senescence phenotype. These results demonstrate that this Toxin Antitoxin module is essential for bacteroid viability (in preparation).

**Conclusions**

We demonstrated the role of two Toxin Antitoxin systems in *S. meliloti* survival during *Medicago sp.* interaction. The two mutants analysed show different phenotypes. To a better understand the role of all VapBC systems; phylogenetic analyses were done to define the evolution origin of these genes.

**FEMS-2001**

**Toxin / antitoxin: activities and functions**

**VAPC FROM THE LEPTOSPIRAL VAPBC TOXIN-ANTITOXIN MODULE  
DISPLAYS SPECIFIC RIBONUCLEASE ACTIVITY ON THE INITIATOR tRNA.  
VAPC HIGH PRESSURE REFOLDING AND IN SILICO STRUCTURAL 3D  
STUDIES.**

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E. Cheng<sup>1</sup>, L. Morgant<sup>2</sup>, E.A.L. Martins<sup>1</sup>

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**Background**

The most accepted hypothesis of the physiological function of the ubiquitous prokaryotic Toxin-Antitoxin (TA) operons is the reversible cessation of cellular growth under stress conditions. *vapBC* operons, present in *Leptospira interrogans*, are classified based on the presence of a ribonucleasic PIN domain in the VapC toxin. Their mechanisms of action remain mostly unknown. The expression of the leptospiral VapC in *E. coli* promotes a strong bacterial growth arrestment, making it difficult to obtain the recombinant protein.

**Objectives**

Produce soluble and active VapC in order to find its target and characterize its activity. Study and rank alternative 3D models of VapC.

**Methods**

*vapBC* locus, *vapB* and *vapC* of *L. interrogans* were cloned and expressed in *E. coli*. Proteins were purified by IMAC after refolding by pressurization (VapC). VapB and VapC interaction was tested by pull-down assay and affinity blotting. Ribonuclease activity was tested towards *E. coli* rRNA and tRNA<sup>fMet</sup>. Models of leptospiral VapC were generated and ranked using SWISS-MODEL.

**Conclusions**

VapC was refolded by high hydrostatic pressure, providing a new method to obtain the active toxin. VapB neutralizes the activity of VapC *in vivo* and *in vitro*. The 3D model of the leptospiral VapC closely matches the Shigella's VapC X-ray structure. In agreement, leptospiral VapC shows no activity towards *E. coli* rRNA and was found to cleave tRNA<sup>fMet</sup>. This finding suggests that the cleavage of the initiator tRNA may

represent a common mechanism to a larger group of bacteria and potentially configures a mechanism of post-transcriptional regulation leading to the inhibition of global translation.

**FEMS-1083**

**Toxin / antitoxin: activities and functions**

## **THE IDENTIFICATION METHOD FOR THE SEQUENCE PATTERN AT THE CLEAVAGE SITES OF MAZF FAMILY ENDORIBONUCLEASES USING MASSIVE PARALLEL SEQUENCING**

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### **Background**

MazF family toxins, representative endoribonucleases that cleave single-stranded RNA (ssRNA) in a sequence-specific fashion, are distributed among bacterial and archaeal lineages. Since their sequence pattern of cleavage sites is highly diverse, they are of importance not only in terms of prokaryotic physiologies, but biotechnological applications.

### **Objectives**

To elucidate physiological roles of MazF family toxins and/or to expand the biotechnological utilization of these endoribonucleases, we established a novel easy-to-use method to effectively determine the pattern of the sequences at their cleavage sites.

### **Methods**

The sequences cleaved by MazF family toxins were determined by a modified RNA-Seq protocol. To evaluate our method, MazF, an ACA specific endoribonuclease derived from *Escherichia coli*, was selected as a model target. Besides, the sequence digested by an uncharacterized MazF homologue from Proteobacteria was also identified using this method. The sequences recognized by these endoribonucleases were further analyzed by using Fluorescence Resonance Energy Transfer (FRET) based assays.

### **Conclusions**

The method developed in this study correctly inferred the sequence pattern at the cleavage sites of the model target, *E. coli* MazF, as ACA. Furthermore, the possible sequence pattern recognized by MazF homologue from Proteobacteria was consistent with the sequence confirmed by FRET based method, indicative that the present method is a useful and straightforward means to explore the potential sequence recognition pattern of these endoribonuclease. This method may be applicable to various toxins that catalyze ssRNA cleavage in a sequence-specific manner, facilitating subsequent physiological investigations and practical uses of these enzymes in the genetic engineering.



## FEMS-1372

### Toxin / antitoxin: activities and functions

#### IN VIVO CHARACTERIZATION OF A BACTERIAL KILLER TYPE IV SECRETION SYSTEM EFFECTOR/IMMUNITY PROTEIN PAIR

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#### Background

Type IV secretion Systems (T4SS) are bacterial nanomachines that transfer proteins across the cell envelope and into target cells. We recently provided the first demonstration that the *Xanthomonas citri* (Xac) T4SS mediates the contact-dependent killing of other bacterial cells [1]. One *Xanthomonas* T4SS effector (X-Tfe) X-Tfe<sup>XAC2609</sup> has *in vitro* lysozyme-like lytic activity. This enzymatic activity is inhibited by the soluble domain of the putative *Xanthomonas* T4SS immunity protein (X-Tfi) X-Tfi<sup>XAC2610</sup>.

[1] D. P. Souza, G. U. Oka, et al., 'Bacterial Killing via a Type IV Secretion System,' *Nat. Commun.*, 2015.

#### Objectives

Here, we set out to investigate the *in vivo* function of X-Tfi<sup>XAC2610</sup>. Since X-Tfe<sup>XAC2609</sup> is predicted to act in the bacterial periplasm and X-Tfi<sup>XAC2610</sup> has a lipobox which directs it to the bacterial outer membrane, the hypothesis is that X-Tfi<sup>XAC2610</sup> acts as an immunity factor against the possibly detrimental autolytic effects of X-Tfe<sup>XAC2609</sup>.

#### Methods

Xac mutants strains carrying single gene deletions ( $\Delta$ X-Tfi<sup>XAC2610</sup>,  $\Delta$ X-Tfi<sup>XAC2609</sup> and  $\Delta$ T4SS) and double mutants ( $\Delta$ X-Tfi<sup>XAC2610</sup>- $\Delta$ T4SS and  $\Delta$ X-Tfi<sup>XAC2610</sup>- $\Delta$ X-Tfi<sup>XAC2609</sup>) were created. Colony phenotypes and cellular viabilities were compared for the above mutant and wild type Xac strains.

#### Conclusions

- 1) X-Tfi<sup>XAC2610</sup> provides immunity against the *in vivo* auto-lytic activity of X-Tfe<sup>XAC2609</sup>.
- 2) The *in vivo* autolytic activity of X-Tfe<sup>XAC2609</sup> does not depend on a functional T4SS.

3) X-Tfi<sup>XAC2610</sup> inhibitory activity is also independent of the Xac T4SS.

FEMS-1329

Toxin / antitoxin: activities and functions

**PLASMID-BORNE MAZE<sup>F</sup> TOXIN-ANTITOXIN LOCI ARE WIDESPREAD IN ANTIBIOTIC-RESISTANT STAPHYLOCOCCUS AUREUS, AND TOXIN ACTIVATION PROVIDES SELECTIVE KILLING**

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**Background**

Antibiotic-resistant bacteria have become a global concern and new strategies to control pathogenic bacteria are urgently needed. In theory, activation of the toxin or inhibition of the antitoxin within a bacterial toxin-antitoxin (TA) system could provide a potent new antibiotic therapy.

**Objectives**

we aimed to identify the location of TA systems, to evaluate the functionality of TA systems in clinical isolates of antibiotic-resistant *S. aureus*, to determine the functionality of TA system and to evaluate the antitoxin as an attractive antimicrobial target for the eradication of antibiotic-resistant *S. aureus*.

**Methods**

To evaluate potential TA loci as therapeutic targets, we screened the plasmid and chromosome sequences of 1000 clinical isolates of *S. aureus* for the presence of TA loci.

Plasmid transformation, ATPase assay, Turbidity and PNA assay were performed.

**Conclusions**

Plasmid-borne *mazEF* TA loci were present in all tested, antibiotic-resistant *S. aureus* strains in Iran and Malaysia. A subset of strains expressed the *mazE* and *mazF* transcripts, and ATPase and growth assays revealed that the *mazEF* TA loci were functional. In addition, the plasmid-borne copies were stable in the absence of antibiotic selection. To activate toxin expression, we targeted the *mazE* antitoxin mRNA using peptide nucleic acid (PNA) oligomers. The anti-*mazE* oligomers were bactericidal against drug-resistant *S. aureus* containing *mazEF* and did not inhibit strains lacking *mazEF*. Therefore, *mazEF* TA loci are widespread in drug-resistant strains of *S. aureus* and are plasmid-borne, and activation of toxin activity by silencing of the antitoxin gene provides a means to selectively kill drug-resistant strains.





**FEMS-1609**

**Toxin / antitoxin: activities and functions**

## **CHARACTERIZATION OF TOXIN-ANTITOXIN SYSTEMS IN THE HUMAN PATHOGEN *STREPTOCOCCUS PYOGENES***

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### **Background**

The Gram-positive bacterium *Streptococcus pyogenes*, also known as group A *Streptococcus* (GAS), is an important human pathogen responsible for a wide spectrum of diseases such as pharyngitis, necrotizing fasciitis, and toxic shock syndrome. In addition to encoding multiple factors for adaptation and pathogenicity, GAS contains several predicted type II toxin-antitoxin (TA) loci. TA systems are widespread in bacterial pathogens and enable bacteria to adapt to rapidly changing environmental conditions, thereby contributing to the pathogenicity of the organisms.

### **Objectives**

To understand the role in physiology and virulence of these putative TAs in *S. pyogenes*, we aim to characterize hypothetical toxin-antitoxins (TAs) and unravel the molecular mechanisms involved in their regulation and mode of action.

### **Methods**

Operon architecture and expression of the predicted loci was analyzed by RT-PCR, RNA sequencing (RNA-seq), and reporter-fusions. Toxin and antitoxin activity and function was assessed by growth arrest assays, activity assays, Western blotting and microscopy.

### **Conclusions**

A series of transcriptional fusions was constructed that enabled the confirmation of TAs annotation. Three out of four predicted TAs cause growth arrest upon overexpression. We show that two of the predicted toxins cause a reduction in CFUs but not in optical density, which can be explained by a cell division defect. Expression of the cognate antitoxins was able to relieve the growth arrests. Understanding the roles of TAs will increase our knowledge on the regulation of bacterial pathogenicity, and may reveal key targets for potential novel antibacterial strategies.



**FEMS-1848**

**Vector-borne pathogens**

## **EXPOSURE TO TICKS AND SEROPREVALENCE OF BORRELIA BURGDORFERI LIVING IN THE PROVINCE OF ERZINCAN, TURKEY**

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### **Background**

Lyme borreliosis is a zoonosis caused by *Borrelia burgdorferi* transmitted by ticks, especially Ixodes species.

### **Objectives**

The aim of this study was to determine the *Borrelia Burgdorferi* seroprevalence caused by ticks in the province of Erzincan, which has a high tick population due to its geographical position and climate conditions.

### **Methods**

368 people, who were live in rural area in the province of Erzincan located in the east of Turkey were included in the study. In all the acquired serum samples, the *B.burgdorferi* IgG antibodies were screened by using the ELISA method (SERION ELISA classic *Borrelia burgdorferi* IgG). The positive and doubtful results obtained were confirmed using the Western blot method (The VIRO-BLOT Anti-Borrelia IgG test).

### **Conclusions**

*B.burgdorferi* IgG antibodies were determined as positive (4.1%) in 15 of all cases and suspicious in 36 cases by using the ELISA method. *B.burgdorferi* IgG levels were examined by using the Western Blot method in totally 51 serum samples that were determined to be positive and doubtful according to the result of the SERION ELISA classic *Borrelia burgdorferi* IgG test. Evaluating all the results together; *B.burgdorferi* IgG positivity was found at the rate of 2.17 % in 8 of 368 cases. The story of tick contact was determined in 3 of cases that were determined to have positive *B.burgdorferi* IgG. *This study proved the presence of Borrelia Burgdorferi in the province of Erzincan. It is thought to offer the preventive health services for those in the risk group in order to minimize the tick exposure.*



## **FEMS-1905**

### **Vector-borne pathogens**

#### **SEROPREVALENCE OF CRIMEAN-CONGO HEMORRHAGIC FEVER LIVING IN THE PROVINCE OF ERZINCAN, TURKEY**

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### **Background**

Crimean-Congo Haemorrhagic Fever (CCHF) is a zoonotic disease caused by a tick-borne CCHF of the genus *Nairovirus* of the family *Bunyaviridae*.

### **Objectives**

The aim of this study was to determine seroprevalence of the CCHF in animal breeders in the province of Erzincan, for which there has been no seroprevalence study despite being accepted as an endemic region in terms of the CCHF by the Ministry of Health.

### **Methods**

372 people, who were live in the province of Erzincan located in the east of Turkey were included in the study. In all the obtained 372 serum samples, the CCHF IgG antibodies were screened by using the ELISA method. The samples that were found to be CCHF IgG positive were examined with CCHF IgM. CCHF IgM-IgG ELISA kits were used in order to determine the antibody levels. IgM positive samples were processed for detection of viral RNA through RT-PCR.

### **Conclusions**

In 13.9% (52/372) of all samples included in the study, the CCHF IgG was determined as positive. This ratio was found respectively as follows; 16.7% (29/174) in people who had a tick contact/bite, 12.4% (18/145) in people who animal breeders and had no tick contact and 9.4% (5/53) in people who were not animal breeders in the city center and had no tick contact. Seven samples were found positive for IgM whereas viral RNA was detected in 1 samples. In the province of Erzincan where many CCHF-related death cases are reported every year, the CCHF IgG antibody level was found to be high as expected.



**FEMS-2636**

**Vector-borne pathogens**

## **CUTANEOUS LEISHMANIASIS IN BADROOD- A HOT POINT OF LEISHMANIOSIS IN ISFAHAN,IRAN**

*M. Momen-Heravi<sup>1</sup>, H. Afzali<sup>1</sup>, H.A. Ahmad<sup>2</sup>, M. Saboori Kashani<sup>3</sup>*

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*<sup>2</sup>Badrood hospital, Isfahan University of Medical Sciences, Badrood, Iran*

*<sup>3</sup>infectious disease, Kashan University of Medical Sciences, Kashan, Iran*

### **Background**

Cutaneous Leishmaniasis(CL) is a common endemic parasitological disease in Iran. This disease is always serious health problems in Esfahan province specially Badrood city and its prevalence has been doubled over the last decade

### **Objectives**

This study was designed to determine the epidemiology and clinical characteristics of CL in Badrood in 2013

### **Methods**

This descriptive cross sectional study was on all detected patients with cutaneous leishmaniasis in the city Badrood by active detection within one year in 2014. Patients were visited by physician and a questionnaire including information about demographic and characteristics of wounds were filled through interview and examination .Diagnosis was confirmed by revealing of leishman body in smear of wounds.

### **Conclusions**

we found 63 definitive case of CL .55.6% of patients were male. Age group 15-30 years had the highest rate (23.8%) among the patients. 66.7% of patients had history of stay in Agha Ali Abbas region. All wounds were rural form. Most patients with cutaneous leishmaniasis in Badrood had a wound with diameter less than 3 cm, secretion, painless, itch and extremities was the most common site for infection

Based on the findings of this study, cutaneous leishmaniasis in Badrood city is rural form and most patient are men and older than 15 year. there was complete or partial healing after treatment in the most patients.



**FEMS-2637**

**Vector-borne pathogens**

## **CUTANEOUS LEISHMANIASIS IN BADROOD- A HOT POINT OF LEISHMANIOSIS IN ISFAHAN,IRAN**

*H.A. Ahmadi<sup>1</sup>, M. Momen-Herav<sup>2</sup>, H. Afzal<sup>2</sup>, M. Saboori Kashani<sup>3</sup>*

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*<sup>3</sup>infectious disease, Kashan University of Medical Sciences, Kashan, Iran*

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This descriptive cross sectional study was on all detected patients with cutaneous leishmaniasis in the city Badrood by active detection within one year in 2014. Patients were visited by physician and a questionnaire including information about demographic and characteristics of wounds were filled through interview and examination. Diagnosis was confirmed by revealing of leishman body in smear of wounds.

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Based on the findings of this study, cutaneous leishmaniasis in Badrood city is rural form and most patient are men and older than 15 year. there was complete or partial healing after treatment in the most patients.

**FEMS-2696**

**Vector-borne pathogens**

## **PREVALENCE OF INTESTINAL PARASITES AMONG PRIMARY SCHOOL PUPILS IN ABAKALIKI METROPOLIS**

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Nigeria*

### **Background**

Intestinal parasites are still major health challenges in tropical and sub-tropical areas. They are commonly found among people with low socio-economic status and in unhygienic environments. Children are usually more infected than adults due to easier fecal-oral transmission<sup>1</sup>. Children with parasitic infections are usually undernourished, weak and may have complications like diarrhoea, nutritional disorders and potentially lethal systemic disease.

### **Objectives**

To determine the prevalence of intestinal parasites among primary school children in Abakaliki metropolis.

### **Methods**

A total of 180 stool samples were aseptically collected and examined macroscopically by wet preparation (saline and iodine) and then microscopically by formal ether concentration techniques. The study was approved by Anambra State University Teaching Hospital Review Board.

### **Conclusions**

Out of the 180 stool samples examined, intestinal parasitic infection prevalence of 25.0% was recorded. Hookworm showed the highest of prevalence, followed by *Ascaris lumbricoides* and *Gardia lamblia*, while *Entaemoba histolytica* had the least prevalence. Prevalence in male was insignificantly higher than that of female ( $p>0.05$ ). The highest prevalence was recorded amongst age group 5-7 years. There was no significant difference in the rate of parasitic infection in relation to age ( $p>0.05$ ). Higher number of parasites was seen in the concentration technique as compared to other laboratory techniques (normal saline and Iodine method). Higher prevalence was seen among the children of farmers compared to other children. A well-structured control programme is needed to reduce mortality and morbidity from intestinal parasites and sustained commitment by the government and policy makers

are needed to improve the quality of life of these children.

**FEMS-0961**

**Vector-borne pathogens**

## **EVALUATION OF HAIR SAMPLES AS TARGET TO DIRECT DETECTION OF LEISHMANIA SPP. IN LEPORIDAE**

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<sup>5</sup>Madrid Salud, Ayuntamiento de Madrid, Madrid, Spain

### **Background**

Leishmaniasis is a parasitic zoonotic disease caused by *protozoa* of the genus *Leishmania*, responsible of high mortality in developing countries and considered one of the three major neglected parasitic diseases in developed countries. Wild reservoirs have a key role in certain epidemiological situations, i.e. *Leporidae* were significantly involved in the largest recorded European outbreak of leishmaniasis, in Madrid (Spain), still active. The possibility of monitoring *Leishmania* spp. using samples easy to obtain, with noninvasive techniques and stable for transport/storage is an essential key for surveillance programs in wild reservoirs. Previous studies have shown that hair samples are ideal for this purpose in dogs, mice and some wild species, but their suitability in wild *Leporidae* has not been studied before.

### **Objectives**

Assessment of hair as a target for direct detection of *Leishmania infantum* in wild *Leporidae*.

### **Methods**

Seventeen wild rabbits and nine hares from Madrid were included. Sera from all the animals were tested by immunofluorescence antibody test (IFAT). Moreover, hair, skin and spleen samples were subjected to DNA extraction and analyzed by real-time PCR for *Leishmania infantum*.

### **Conclusions**

All animals sampled (n=26) were positive to at least one of the three classic samples (serum, skin, spleen): 23 individuals (88.5%, 95%CI: 77.3%-100%) were positive in IFAT and 25 (96.2%, 95%CI: 88.2%-100%) were positive in skin/spleen RT-PCR.

A total of 22 hair samples (84.6%, 95% CI: 69.7%-99.8%) were positive. Despite the limited sample size, our results demonstrate that hair could be considered an adequate sample for direct diagnosis of *Leishmania infantum* infection in wild *Leporidae*.

**FEMS-1041**

**Free subjects - 2**

**A NEW SPECIES OF CHOLEOEIMERIA (APICOMPLEXA: EIMERIIDAE)  
PARASITIC IN THE ROUGH-TAILED GECKO CYRTOPODION SCABRUM  
(SAURIA: GEKKONIDAE) IN EGYPT**

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**Background**

*Cyrtopodion* Fitzinger is a large genus of the Gekkonidae Gray (gecko) family with currently 28 described species. In contrast to the large diversity of this genus, only *Eimeria lineri* McAllister et al. 1988 has so far been described. In Egypt, the genus *Cyrtopodion* is represented only by *Cyrtopodion scabrum* Heyden 1827. Genus *Choleoeimeria* Paperna and Landsberg 1989 was proposed to incorporate eimeriid-like coccidia of elongate-ellipsoidal oocysts (L/W ratio 1.6–2.2), with sporocysts that lack a Stieda body and that develop in the epithelium of the gall bladder and bile duct of their hosts.

**Objectives**

The present work aims to establish the taxonomic status of an unknown *Choleoeimeria* from the gallbladder of the rough-tailed gecko *Cyrtopodion scabrum* using the characteristics of exogenous and endogenous stages of the parasite

**Methods**

A total of 15 rough-tailed geckoes, *Cyrtopodion scabrum* were collected and examined for coccidian infection. The presence of oocysts was determined by direct microscopic examination of bile contents. To study the endogenous stages, infected gallbladders were fixed in 10% neutral buffered formalin and embedded in paraffin. Sections were cut and stained with haematoxylin and eosin (H&E).

**Conclusions**

Based on the combination of the oocyst morphology, site and characters of the endogenous development; we assigned the present species to the genus *Choleoeimeria* and to which the name *Choleoeimeria scabrumi* n. sp. was suggested.

**FEMS-0627**

**Free subjects - 2**

## **BACTERIAL CONTAMINATION OF TOYS AND PLAY AREAS IN RESTAURANTS AND SHOPPING MALLS IN RIYADH, SAUDI ARABIA**

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<sup>2</sup>*College of Medicine, King Saud Bin Abdulaziz University For Health Sciences, RIYADH 11323, Saudi Arabia*

### **Background**

This study was conducted to determine the load of pathogenic bacterial contamination and explore the prevalence of pathogenic bacterial species in play areas of fast food restaurants and shopping malls in Riyadh, Saudi Arabia. This novel study aims to pilot other studies in the area to explore bacterial contamination for purposes preventing pediatric disease

### **Objectives**

The study seeks to identify and determine the load and antibiotic sensitivity of bacterial organisms in the play areas present in fast food restaurants and shopping malls in Riyadh, Saudi Arabia.

### **Methods**

The sample included three fast food restaurants' play pits and two shopping mall play areas in Riyadh, Saudi Arabia during the month of March in 2012. Nineteen samples were isolated using sterile swabs from surfaces of balls in ball pits and different arcade games. Samples were inoculated onto blood agar and MacConkey agar and were incubated 24 hours at 37° C. Bacterial species were identified and tested for their antibiotic sensitivity. Bacteria isolated from a total of 19 samples included *Klebsiella pneumoniae*, *Staphylococcus spp.*, Diphtheroids, *Pantoea agglomerans*, *Enterococci spp.*, *Streptococci viridans* and two fungal growths that included *Aspergillus spp.* and *Penicillium spp.*

### **Conclusions**

The results of this study confirm that toys in play areas and arcades could serve as media for the transmission of pathogenic microorganisms and fungal elements that pose a potential health risk for children.





**FEMS-3054**

**Free subjects - 2**

**EFFECTS OF ORAL ADMINISTRATION OF THE BUTYRIVIBRIO GROUP BACTERIUM ON THE INFLAMMATORY DRY SKIN CONDITION IN CHRONIC IRRITANT CONTACT DERMATITIS**

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<sup>1</sup>Life Science, Meiji University, Kawasaki, Japan

**Background**

The genus *Butyrivibrio* is one of the predominant residents in the rumen, and also inhibits the intestine of monogastric animals. We previously reported that oral administration of *B. fibrisolvens* to mice alleviated experimental enterocolitis and suppressed the formation of colorectal aberrant crypt foci. Although the preventive mechanisms of this bacterium have been still unknown, it might have the potential to improve immune responses. Thus, some strains of this bacterial group might be used for improving the dysfunction in inflammatory skin diseases such as irritant contact dermatitis (ICD).

**Objectives**

The newly strain of *Butyrivibrio* group to improve the skin barrier function was isolated and its effect on inflammatory dry skin was examined.

**Methods**

Several strains of the *Butyrivibrio* group bacteria were isolated from the goat rumen and confirmed by reading the nearly full-length 16S rRNA gene sequences. Some strains were examined as the candidates for probiotic bacteria that recover the skin barrier function. Chronic ICD was induced to mice with oxazolone at the ears by repeated application. The isolated strains were administrated as the freeze-dried cells. The beneficial effects on cutaneous inflammation were evaluated by the dermatitis severity score, lymph node weight, histological analysis, and mRNA expression of IL-1 $\beta$  and IL-6 in the lesional ear.

**Conclusions**

When the isolated strain was administrated, the development of skin roughness or exfoliation was alleviated and mRNA expression of the pro-inflammatory cytokines was suppressed. In conclusion, this newly isolated *Butyrivibrio* strain might be used as a probiotics for the restoring the skin barrier function.

**FEMS-2278**

**Free subjects - 2**

**EMULSION OF THE AMAZON FOR DENTAL CAVITY CLEANING: PHYSICO-CHEMICAL QUALITY CONTROL AND EVALUATION OF ANTIBACTERIAL ACTIVITY**

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M.C. Vasconcelos<sup>3</sup>, L.G. Moura<sup>3</sup>, L.R. Machado<sup>3</sup>, N.C.O. Conde<sup>1</sup>

<sup>1</sup>Dental School, Amazonas Federal University, Manaus, Brazil

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<sup>3</sup>Pharmacy School, Amazonas Federal University, Manaus, Brazil

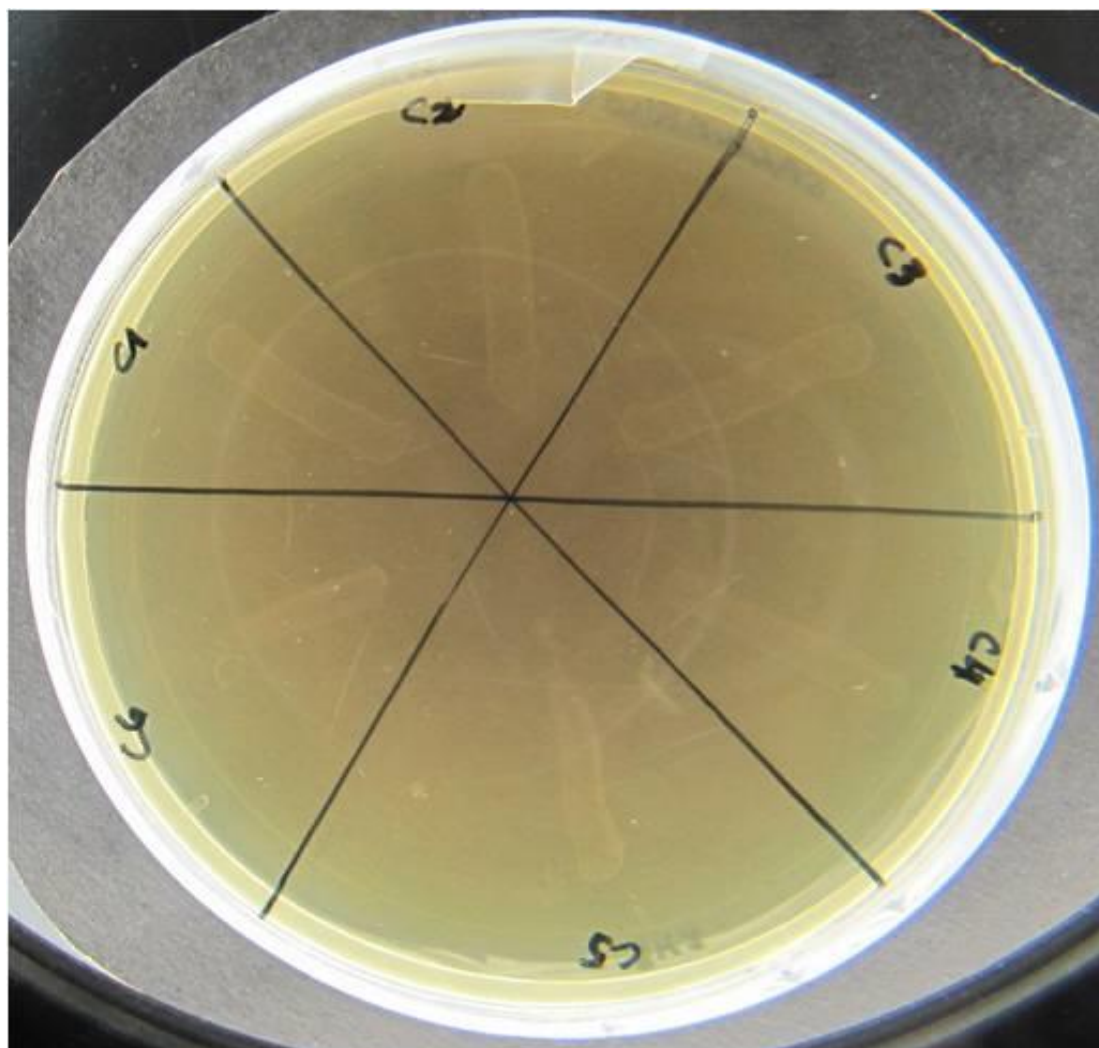
**Background**

The dental field has been developed with the purpose of seeking new bioactive principles for the formulation of drugs with different applicability.

**Objectives**

Analyze the stability of an emulsion of copaiba oil (*Copaifera multijuga* – CM) for dental cavity cleaning. In this emulsion was performed physico-chemical tests and antibacterial test in different environments and periods.







### Methods

The results of the pH and density determination were analyzed by ANOVA and Bonferroni's test ( $p < 0,05$ ). For other tests were given descriptive analysis. In the centrifugation, there was no phase separation at time 0, in 3 months (freezer, refrigerator and air conditioning) and in 6 months (in the freezer and refrigerator) ; in the pH, the incubator, the room temperature protected from light, the room temperature and the air conditioning had statistically significant difference from the control group (time 0); at density, the incubator had the lowest density values; in microbiological evaluation, there was no bacterial growth; for the evaluation of organoleptic characters, there was only change in color of the emulsion in 3 months (incubator) and 6 months ( incubator , room temperature protected from light and room temperature). The CM showed bacteriostatic and bactericidal activity, respectively, in concentrations of 13.33 $\mu$ L/ml and 15  $\mu$ L/ml for *S. mutans*; 20  $\mu$ L/ml and 23.33  $\mu$ L/mL for *S. salivarius* and 13.33  $\mu$ L/ml, 15  $\mu$ L/ml for *L. paracasei*; 8.33 $\mu$ L/ml and 8.33 $\mu$ L/ml for *S. oralis*.

### Conclusions

The authors concluded that the CM demonstrated bactericidal activity; presented conditions of stability and quality without contaminants, the best storage environment is refrigerator.



**FEMS-2452**

**Free subjects - 2**

**A CASE OF CHRONIC OTITIS MEDIA DUE TO ACHROMOBACTER  
XYLOSOXIDANS IN AN IMMUNOCOMPETENT PATIENT**

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Gulhane Military Medical Academy Haydarpasa Training Hospital, Istanbul, Turkey

**Background**

*Achromobacter xylosoxidans* is an aerobic, oxidase positive, non-fermentative, gram-negative bacillus and often misidentified as *Pseudomonas aeruginosa* by its oxidase positive feature. It may cause serious nosocomial and community-acquired infections especially in patients with immune deficiency.

**Objectives**

We present a case of chronic otitis media due to *A.xylosoxidans* which is rarely isolated from clinical materials in routine microbiology laboratory practice. In this report we emphasize clinical impact of *A.xylosoxidans* and its antimicrobial resistance profile.

**Methods**



A 20-year-old immunocompetent man was admitted to otorhinolaryngology service of our hospital with the complaints of left ear pain, difficulty in hearing, left ear discharge beginning from nearly three months his admission. His history revealed unsuccessful treatment attempts with various oral antimicrobials with the diagnosis of otitis media. Tympanic membrane perforation and mucopurulent yellow discharge was observed. He had no systemic symptoms. Microscopic examination of the Gram stained smear revealed PMNL and gram negative bacilli. *A.xylosoxidans* was isolated from discharge. Identification was performed by VITEK 2 automated system. Antimicrobial susceptibility was detected by disc diffusion method according to CLSI criteria for *Pseudomonas* spp. It was resistant to penicillins, most of the beta lactamases, fluoroquinolones, aminoglycosides and susceptible to piperacillin-tazobactam and imipenem.

## Conclusions

*A.xylosoxidans* strains are often multidrug resistant. Empirical antibiotic therapy with piperacillin-tazobactam or a carbapenem is a reasonable choice until the results of susceptibility tests are available. It should be kept in mind that even an opportunistic and generally nosocomial pathogen like *A.xylosoxidans* can cause community-acquired infection like otitis media in a immunocompetent host.

FEMS-1710

Free subjects - 2

## EFFECT OF TRANS-CHALCONE ON TRANSCRIPTION OF GENES RELATED TO CELL WALL AND FATTY ACID SYNTHESIS IN THE DERMATOPHYTE TRICHOPHYTON RUBRUM

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### Background

*Trichophyton rubrum* is the main causative agent of dermatophytosis worldwide. The infections caused by this dermatophyte are not lethal but are uncomfortable and difficult to treat. Moreover, invasive infections are rising due to modern medical interventions and immunosuppressive diseases. The development of fungal resistance limits the arsenal of antifungal drugs, which has a narrow spectrum of activity and often presents toxicity to the host. Therefore, novel drugs with more specific and effective mechanisms of action against dermatophytes are urgently needed. Chalcones drew attention by numerous pharmacological applications, including antifungal activity.

### Objectives

The aim of this study was to evaluate the modulation of genes involved in cell wall and fatty acid synthesis of *T. rubrum* after the exposure to *trans*-chalcone.

### Methods

The modulation of fatty acid synthesis genes (FAS 1p and FAS 2p subunits, acetyl-CoA carboxylase 2p subunit) and genes of cell wall synthesis (beta-1,3-glucanosyltransferase and glycosylphosphatidylinositol anchored protein) was checked by qPCR after the exposure to MIC (7.8 µg/mL) and ¼ MIC (1.95 µg/mL) of *trans*-chalcone, respectively, for 16h for fatty acid genes and 7h for cell wall genes.

### Conclusions

*Trans*-chalcone promoted a down-regulation of genes of fatty acid synthesis whereas cell wall genes were up-regulated. These results suggest that *trans*-chalcone may act on different fungal specific targets, which could be considered an advantage for an antifungal compound.



## REP-PCR GENOMIC FINGERPRINTING AND NEW INSIGHTS INTO PHYSIOLOGICAL TRAITS OF ACIDIPLASMA STRAINS

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### Background

*Acidiplasma* is the genus of acidophilic, ferrous iron-oxidizing, heterotrophic, cell wall-lacking, moderately thermophilic microorganisms belonging to the family *Ferroplasmaceae*. All representatives of the genus have high similarity of 16S rRNA gene (99-100%). The genus includes two species (*A. aeolicum* and *A. cupricumulans*) separated by DNA–DNA hybridization (DDH) values. However complexity of DDH suggests to necessity of application of alternative tools for distinguishing representatives within the genus. Also information about physiological traits of the genus may be incomplete. In our previous study the new physiological trait (ability to oxidize elemental sulfur) was shown by the example of strain *Acidiplasma* MBA-1.

### Objectives

The aims of this study were to apply rep-PCR fingerprinting technique for distinguishing *Acidiplasma* representatives and to investigate sulfur oxidation by the strains *A. aeolicum* V<sup>T</sup>, *A. cupricumulans* BH2<sup>T</sup>, *Acidiplasma* MBA-1, *Ferroplasma acidiphilum* Y<sup>T</sup>.

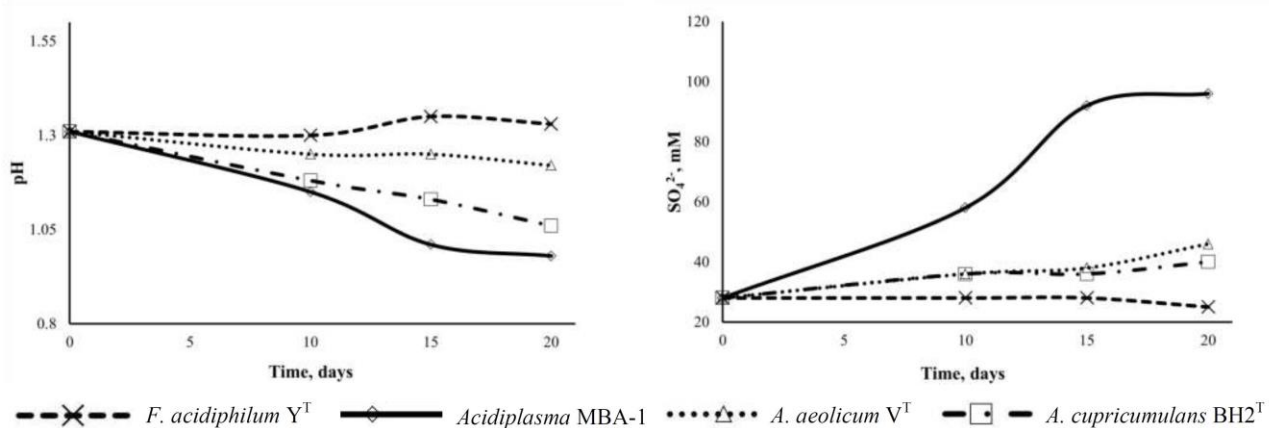
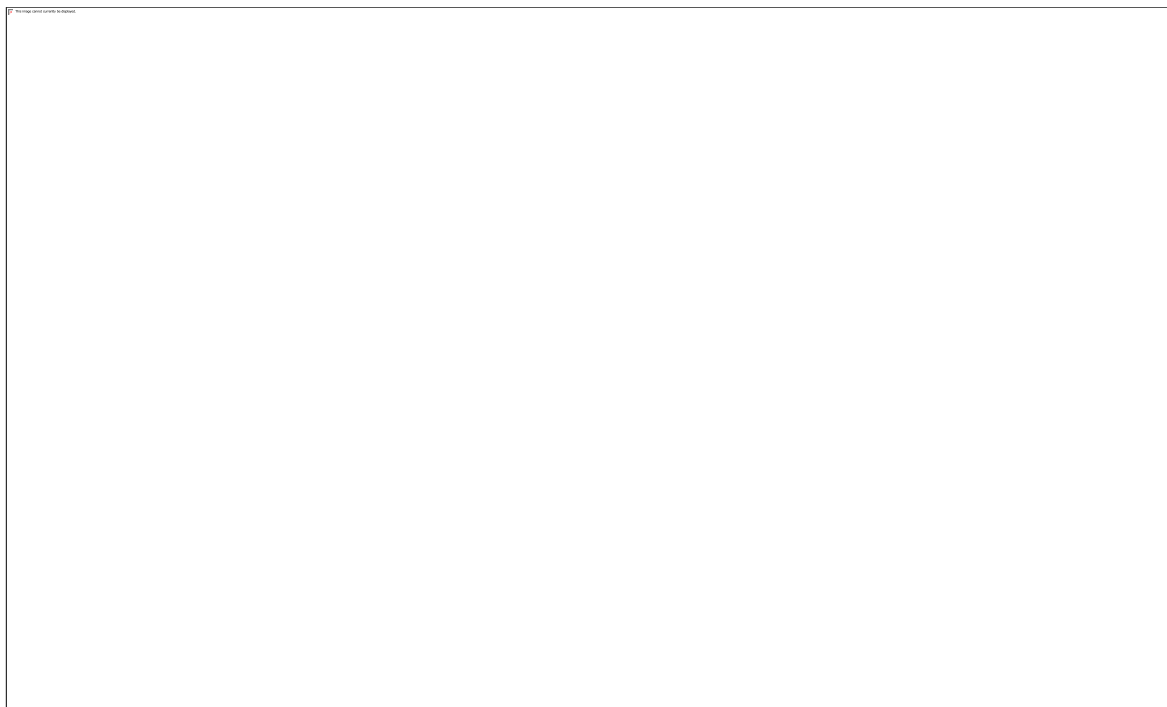


Figure 1. Oxidation of sulfur by *Acidiplasma* strains and *Ferroplasma acidiphilum* Y<sup>T</sup>



## Methods

The genomic fingerprints were obtained using BOXA1, BOXS1, ERIC and REP primers. Oxidation of sulfur was estimated by pH decreasing and increasing of sulfates-ions concentrations.

## Conclusions

Sulfur was oxidized by all *Acidiplasma* strains, but not by *F. acidiphilum* Y<sup>T</sup> (Fig. 1). It suggests sulfur oxidation can be widely distributed among *Acidiplasma* representatives, but not among *Ferroplasma* representatives. Each of *Acidiplasma* strains possess its unique genomic fingerprint (Fig. 2), it suggests genomic fingerprinting could be rapid, highly discriminatory screening techniques for *Acidiplasma* representatives. The work was supported by the Russian Foundation for Basic Research, project no. 14-04-31210 mol\_a and Core Research Facility 'Bioengineering' equipment.

**FEMS-2995**

**Free subjects - 2**

## **TREATMENT AGAINST THE MICROBIAL ACTIVITY WITH CINNAMON ESSENTIAL OIL IN ARTISTIC WORKS AND PACKAGING**

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<sup>2</sup>CONSERVACIÓN, MACBA, Barcelona, Spain

### **Background**

The artwork and the packaging system used in all transfers and storage is a good substrate for the growth of microorganisms that can produce irreversible changes, as destruction or alteration of substrates on which microorganisms can multiply and that as a result of secondary metabolism can accumulate among them the indelible pigment

### **Objectives**

The aim of this study is to provide a preventive solution to the development of microorganisms without risk to the integrity of the artwork.

### **Methods**

We proceeded through sterile swabs to collection of samples from artwork and packaging deposited in the MACBA. Samples were plated on TSA to determine the presence of bacteria and AS with antibiotic to determine fungi.

All artwork and packaging were submit an aspiration synthetic pallet and vacuum equipped with a HEPA filter.

Then, we applied by spraying a mixture of alcohol: water: cinnamon and elsewhere , to compare the results for the traditionally used mixture formed alcohol: water.

After a month of the application of the products we collected samples from all the treated areas in order to evaluate the efficacy of treatments. In order to assess the effect of treatment in cases of contamination, proceeded experimentally inoculated packaging materials and pieces of artwork who underwent both treatments indicated and to expose the action of an environment contaminated items artistic and packaging that did not have initially any pollution.

### **Conclusions**

The results indicates that the mixture comprising cinnamon oil has a strong antimicrobial effect and avoid contamination of sensitive material in contact with elements altered by microorganisms.

**FEMS-2424**

**Free subjects - 2**

## **THE EFFICACY OF PRtA TO PREVENT STREPTOCOCCUS PNEUMONIAE INFECTION**

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<sup>2</sup>*Research Center of Bacterial Pathogenesis, Chang Gung University, Taoyuan, Taiwan*

### **Background**

*Streptococcus pneumoniae* is a respiratory pathogen contributing to severe lung infection and/or bacteremic complications. Worldwide efforts were made to develop a conserved protein vaccine against *S. pneumoniae* and to provide a maximal and memory protection.

### **Objectives**

The pneumococcal surface protein, PrtA, was identified by convalescent patient serum screening on a pneumococcal genomic expression library. The *prtA* gene is prevalent and conserved among *S. pneumoniae* strains; however, there was no report describing its protective efficacy.

### **Methods**

Since IL-17A elicitation can improve the clearance of pneumococcal colonization, we combined a recombinant PrtA fragment (aa 144-1041) with curdlan, a strong inducer of IL-17A, to vaccinate three-wk old CD1 mice intranasally once a week for three weeks. It showed an elevated PrtA-specific Ab titer in mucosa and increased IL-17A production by splenocytes compared with sham group. Finally, the PrtA vaccination can successfully reduce *S. pneumoniae* D39 colonization in lung within 24 hr but failed to reduce the mortality after 12 days of infection. All of the experiments were approved by the Institutional Animal Care and Use Committee of the National Defense Medical Center Laboratory Animal Center. The animal experiments were performed in strict accordance with the Taiwan regulations of the Animal Protection Act and the course on Animal Care and Use in Research and Education from the American Association for Laboratory Animal Science.

### **Conclusions**

Combined with PrtA fragment and curdlan adjuvant can enhance PrtA-specific humoral and cellular immunity; however, the vaccination was not sufficient to protect against *S. pneumoniae* infection.

**FEMS-0835**

**Free subjects - 2**

## **ANTI-TOXOPLASMA ACTIVITIES OF METHANOLIC EXTRACT FROM ZEA MAYS AND ERYNGIUM CAUCASICUM**

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### **Background**

*Toxoplasma gondii* is an obligatory intracellular parasite that infects a wide range of warm-blooded animals and humans.

### **Objectives**

Considering the severity of toxoplasmosis, side effects of current treatments, and the contribution of the ethnopharmacological knowledge for the treatment of parasitic infections, the aim of the present study was to investigate the efficacy of methanolic extracts of *Zea mays* and *Eryngium caucasicum* against tachyzoite of *T. gondii*.

### **Methods**

Four concentrations (5, 10, 25 and 50 mg ml<sup>-1</sup>) of extracts were incubated with infected macrophages for 30, 60, 120 and 180 min and then the viability of the tachyzoites were evaluated by trypan blue staining.

### **Conclusions**

Concentrations of 10 and 25 mg ml<sup>-1</sup> of *Z. mays* after 180 minutes, and also concentration 50 mg ml<sup>-1</sup> after 120 minutes killed 100% of the tachyzoites. Also high anti-*Toxoplasma* activity was seen using *E. caucasicum* extract. The anti-*Toxoplasma* effects of the methanolic extracts from *Z. mays* and *E. caucasicum* did not show any significant difference in comparison with pyrimethamine (positive control). Findings of this research indicate that these extracts have acceptable anti-*Toxoplasma* efficacies *in vitro* and it can be used as candidate extracts for further research on treatment of toxoplasmosis *in vivo*.

**FEMS-0836**

**Free subjects - 2**

**THE INHIBITORY EFFECT OF CROMOLYN SODIUM AND KETOTIFEN ON  
TOXOPLASMA GONDII ENTRANCE INTO HOST CELLS IN VITRO AND IN VIVO**

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**Background**

*Toxoplasma gondii* is a protozoan with worldwide distribution and in spite of increasing information about its biology, treatment of toxoplasmosis is restricted to a few drugs and unfortunately using of each of drugs is associated with significant side effects in patients.

**Objectives**

This study was designed to evaluate the efficacy of cromolyn sodium and ketotifen as alternative drugs for the treatment of toxoplasmosis.

**Methods**

In vitro; in case group, concentrations of 1, 5, 10 and 15 lg/ml of ketotifen and cromolyn sodium were added to RPMI medium containing peritoneal macrophages. After 1 h incubation and adding tachyzoites to medium, efficacy rate of these drugs in entrance inhibition of *Toxoplasma* tachyzoites into macrophages were evaluated after 30 and 60 min. In vivo; case groups received ketotifen and cromolyn sodium with different concentrations at various times. Control groups received none of drugs.

**Conclusions**

We found that in vitro; after 60 min the best efficacy of these drugs in inhibition of cell entrance of *Toxoplasma* was observed at 15 lg/ml ( $78.9 \pm 1.70$  and  $91.97 \pm 0.37$  %, respectively) ( $P \leq 0.05$ ). In vivo; after 60 min ketotifen at 2 mg/kg in 3 h before tachyzoite injection ( $69.83 \pm 2.25$  %), and cromolyn sodium, at 10 mg/kg in 6 h before tachyzoite injection ( $80.47 \pm 2/49$  %) had the

best effect on inhibition of *Toxoplasma* entry into the cells ( $P \leq 0.05$ ). Our findings show that ketotifen and cromolyn sodium are suitable drugs for entrance inhibition of tachyzoites into nucleated cells in vitro and in vivo.



**FEMS-1738**

**Free subjects - 2**

**THE NAGOYA PROTOCOL: REVOLUTION OR EVOLUTION? ACCESS AND STUDY OF MICROBIAL DIVERSITY IS SUBJECT TO NEW REGULATION**

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**Background**

The Nagoya Protocol<sup>[i]</sup> (NP) under the Convention on Biological Diversity (CBD) has entered into force on 12 October 2014. The NP covers the use of biological diversity in Research and Development. If not correctly translated by lawmakers or inefficiently apprehended by microbiologists it could heavily impact the activities of Culture Collections (CC) and their partners<sup>[ii]</sup>. The European lawmakers have already translated the NP into European Regulation<sup>[iii]</sup>.

[i] Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization to the Convention on Biological Diversity. (<http://www.cbd.int/abs/> )

[ii] Fidler, D.P. (2008). Influenza Virus Samples, International Law and Global Health Diplomacy. *Emerg. Infect Dis* Jan 2008 14(1): 88-94.

[iii] Regulation (EU) No 511/2014 of the European Parliament and of the Council of 16 April 2014 on compliance measures for users from the Nagoya Protocol on Access to Genetic Resources and the Fair and Equitable Sharing of Benefits Arising from their Utilization <http://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32014R0511&from=EN>

**Objectives**

The challenge is to organize efficiently the access and the conveyance of transfer and utilization of the microorganisms at affordable cost.

**Methods**

CCs have anticipated these legal developments and have presented solutions during the 1st NP's Meeting of the Parties.



## **Conclusions**

CCs propose TRUST<sup>[i]</sup>, a system to help microbiologists dealing with this new set of rules on access and utilization of biological diversity, including micro-organisms.

[i] Implementing the Nagoya Protocol in microbiology - Gaining trust, building TRUST.  
Note of the World Federation for Culture Collections  
(<http://www.cbd.int/doc/meetings/abs/np-mop-01/information/np-mop-01-inf-08-en.pdf>  
)

**FEMS-2310**

**Free subjects - 2**

## **TREHALOSE METABOLISM IN SACCHAROMYCES CEREVISIAE DURING HEAT STRESS**

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### **Background**

*Saccharomyces cerevisiae* trehalose synthesis complex is composed by Tps1 and Tps2 and two regulatory subunits, Tsl1 and Tps3. To protect membranes trehalose must be present on both sides of the bilayer.

### **Objectives**

Our goal was to elucidate the protective mechanism of trehalose.

### **Methods**

The absence of Tsl1 abolished the increase in Tps1 activity and accumulation of trehalose in response to a 40°C treatment, whereas deficiency of Tps3 only reduced Tps1 activity and trehalose. In extracts of heat stressed cells, Tps1 was inhibited by trehalose-6-phosphate and by cAMP. In contrast, cAMP-dependent phosphorylation did not inhibit Tps1 in *tps3* cells, which accumulated a higher proportion of trehalose-6-phosphate after heating. Agt1 deficiency led to a reduced tolerance to heat shock, without interfering in trehalose accumulation, probably because the outside of the lipid bilayer was unprotected. *ath1* cells still showed a high trehalose levels when they were shifted back from 40 to 28°C, as well as, increased tolerance to a subsequent heat stress.

### **Conclusions**

We conclude that Agt1 plays a crucial role in transporting endogenous trehalose to the outer side of the plasma membrane when yeasts face adverse conditions. With the end of the stress, the intracellular trehalose pool would be hydrolyzed by Nth1 whereas the external molecules would be hydrolysed by Ath1. To stop readily trehalose synthesis during stress recovery, Tps3 would be phosphorylated by cAMP-dependent protein kinase, decreasing Tps2 activity and, consequently, increasing trehalose-6-phosphate levels which, in turn, would inhibit Tps1. We believe that these concepts are of great importance for medical and biotechnological applications.

**FEMS-1072**

**Free subjects - 2**

## **INVESTIGATION OF ROSMARINUS OFFICINALIS L EXTRACT ON THE BACTERIA CAUSES GENITAL INFECTION**

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### **Background**

Some microbial causes in creating vaginitis are *streptococcus group B* and *Gardnerella vaginalis* which result in irrecoverable harms and increasing fatality, and providing infection in pregnancy period and transfer to the baby.

### **Objectives**

the antibacterial effects some plants like Rosemary (*Rosmarinus officinalis*) on vaginitis we can identify these kinds of plants and then give them to the people in the form of plant drugs. This research is an investigation of Rosemary and its antibacterial effect on the bacteria and also comparing this effect with common antibacterial.

### **Methods**

This study of 96 women who suffered from this disease, all samples confirms with phenotyping method. The Disk diffusion method was used for comparing the effect of antibacterial total extract with common antibiotics.

### **Conclusions**

The MIC result for each strain showed respectively; *streptococcus* 1/64, *Listeria* 1/128, *Candida Albicans* 1/2, *staph aureus* 1/128, *Gardenerella vaginalis* 1/64. In the disk diffusion method, the results were in this form which *streptococcus group B*, *staph aureus* and *Gradenerella vaginalis* were more sensitive than antibiotic disk in comparing with extract, While *Listeria monocytogenes* was more sensitive than antibiotic disk to the extract.

**FEMS-1294**

**Free subjects - 2**

## **CHEMICAL FUSION OF PLEUROTUS OSTREATUS AND SACCHAROMYCES CEREVISIAE PROTOPLASTS**

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### **Background**

Lignin is one of the major components of plant cell wall and has significant impact on animal digestion of plants. It is known that Phanerochaete secretes a series of extracellular oxidases, which are encoded in multiple genes, to degrade lignin. However, Phanerochaete needs more stringent conditions to ferment. For animal husbandry application, it is challenge in genetic engineering to transfer all required oxidase genes from Phanerochaete into other strains that can ferment easily. Therefore, we chemically fuse protoplasts between *Pleurotus ostreatus*, a Phanerochaete strain, and *Saccharomyces cerevisiae*. To gain strains with the excellent characteristics of both *Pleurotus ostreatus* and *Saccharomyces cerevisiae*.

### **Objectives**

We chemically fuse protoplasts between *Pleurotus ostreatus*, a Phanerochaete strain, and *Saccharomyces cerevisiae* to gain strains with the excellent characteristics of both *Pleurotus ostreatus* and *Saccharomyces cerevisiae*.

### **Methods**

At first, the enzymatic composition, duration and temperature of emzymolysis, the types of osmotic stabilizer and the age of strains were screened to gain optimal protoplasts preparative condition through L<sub>16</sub> (4<sup>5</sup>) orthogonal test. Next, *Pleurotus ostreatus* and *Saccharomyces cerevisiae* protoplasts were inactivated by heat and UV, respectively. Duration and temperature of inactivation were screened for optimal conditions. Then, the optimal chemical fusion conditions, including fusion temperature and duration, pH value, Ca<sup>2+</sup> concentration and polyethylene glycol-4000 concentration, were screened through L<sub>16</sub> (4<sup>5</sup>) orthogonal test. At last, antagonism, morphological comparison of hypha and Random Amplified Polymorphic DNA were used to characterize the potential fusants, which was analyzed using NTSYS-PC software.

## Conclusions

183 regenerative strains were obtained and, among them, 14 strains were proved to be acquired from fusing *Pleurotus ostreatus* and *Saccharomyces cerevisiae* protoplast.

**FEMS-0832**

**Free subjects - 2**

## **ARE DOG ENZYMES INVOLVED IN THE FORMATION OF THE YEAST TPS2 DELETION MUTANT'S RESIDUAL TREHALOSE?**

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### **Background**

Trehalose is a disaccharide of which the concentration correlates very well with cellular stress resistance in yeast. It is synthesized in two enzymatic steps, where the intermediate trehalose-6-phosphate (Tre6P) is converted to trehalose by the enzyme Tps2. However, deletion of this gene does not completely abolish trehalose production. A possible explanation for this observation is that unspecific sugar phosphatases take over the function of the Tre6P phosphatase in a *tps2Δ* mutant. Screening the *Saccharomyces cerevisiae* proteome for enzymes containing the phosphohydrolase motifs present in Tps2 identified Dog1 and Dog2 as possible candidates. These enzymes are known to dephosphorylate 2-deoxyglucose-6-phosphate, but the natural substrate remains unknown.

### **Objectives**

Are Dog1 and Dog2 tre6P-phosphatase that are active in the absence of Tps2?

### **Methods**

We generated *S. cerevisiae* *DOG1* and *DOG2* deletion and over-expression mutants in the wild type and a *tps2Δ* background and determined heat stress tolerance and trehalose levels in these strains.

### **Conclusions**

The results obtained with the deletion strains seem to support our hypothesis. The *dog1Δ dog2Δ tps2Δ* mutant is more sensitive to heat stress than the *tps2Δ* mutant, which would indicate lower trehalose levels in the absence of the *DOG* genes. However, the results obtained with the *DOG1* and *DOG2* over-expression strains do not support the hypothesis, as trehalose levels and Tre6P-phosphatase activities are not increased in these strains. These results seem to indicate that the Dog1 and Dog2 may be involved in the expression of trehalose metabolism genes or may affect the enzymatic activity of these genes.

**FEMS-2967**

**Free subjects - 2**

## **MYCELIUM AS BIO-BASED MATERIAL**

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### **Background**

One of the challenges of this century is to transform our economy into an eco-friendly and self-sustaining system. An innovative approach is the use of mycelium based materials.

### **Objectives**

The mycelium of the mushroom forming fungus *Schizophyllum commune* has great potential for developing such materials.

### **Methods**

Mycelium of *S. commune* was grown as a floating layer on liquid medium in the light or in the dark under ambient or a high concentration of CO<sub>2</sub>. Mycelia were dried and subjected to increasing tensile force. Their tensile modulus (E), elongation (ε) and stress of the sample at breaking point (σ) were measured. These values were rather low for the wild-type strain 4-39 that had been grown in the light at low CO<sub>2</sub> (E= 0.03±0.02 GPa, ε=0.75±0.28 %, σ: 0.44±0.31 MPa), resembling a crumble material that breaks easily. The properties of the wild-type strain 4.8b that had been grown under the same conditions resembled a flexible material (E: 0.22±0.08 GPa, ε=1.8±0.56 %, σ=3.5±0.9 MPa). Strain 4-39 and 4.8b grown in the light at high CO<sub>2</sub> had plastic-like and even stiffer properties, respectively (4-39: E=0.26± 0.12 GPa, ε= 1.15±0.49 %, σ=point 2.59±1.30 MPa; 4.8b: E=0.50±0.10 GPa, ε =1.13±0.21 %, σ= 5.58±1.65 MPa). Similar results were obtained with 4.8b and 4-39 when grown in the dark, regardless of CO<sub>2</sub> concentration.

### **Conclusions**

Taken together, physical properties of the mycelium is strain dependent and are affected by environmental growth conditions.

**FEMS-3107**

**Free subjects - 2**

## **CHARACTERIZATION OF AIF5A (AEF5): AN EVOLUTIONARY CONSERVED TRANSLATION FACTOR**

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### **Background**

Protein synthesis is a complex process of fundamental importance in all cells during which a nucleotide sequence is translated into an amino acid sequence. The translation apparatus includes mRNA, ribosomes, aminoacyl-tRNAs and a number of translation factors, which assist all stages of the process. Some of the translation factors are conserved among Bacteria, Archaea and Eukarya.

One of the universally conserved factors is eIF5A, highly conserved between Eukarya and Archaea, with a orthologue (EF-P) in bacteria. It was initially identified as a translation initiation factor, but recent studies have established a role for both eIF5A and EF-P in translation elongation specifically in promoting the translation of polyproline containing proteins. The two proteins are both characterized by particular post-translational modifications, which are essential for their function: hypusination for eIF5A and lysinylation for EF-P.

### **Objectives**

Eukaryal and bacterial proteins have been extensively studied, while information on the archaeal homologues are extremely limited so in order to fill this gap we have started the characterization of the archaeal protein in *Sulfolobaceae*.

### **Methods**

We have produced the recombinant protein from *S. solfataricus* using two different expression systems: *E. coli* and *S. acidocaldarius* and the purified protein was then used in several *in vitro* functional tests.

### **Conclusions**

Results clarifying its function in the translation process will be presented and a molecular modelling will shed light on its interaction with the ribosome. In addition, we failed to obtain a knock-out strain in *S. acidocaldarius* proving that, as for the eukaryal and bacterial proteins, the gene product aIF5A is essential.



**FEMS-2168**

**Free subjects - 2**

**AN INVESTIGATION OF BURKHOLDERIA CENOCEPACIA  
LIPOPOLYSACCHARIDE (LPS) MODIFICATION DURING CYSTIC FIBROSIS (CF)  
CHRONIC INFECTION AND ITS IMPACT ON HOST RESPONSE MODULATION**

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**Background**

Bacterial species within the CF microbiome demonstrate adaptive strategies including altered expression of LPS. The structure of LPS governs its immuno-stimulatory potential. It is thus important to elucidate how LPS is modified during chronic infection and to determine its effects on host response modulation.

**Objectives**

We are investigating LPS extracted from four *B. cenocepacia* sequential isolates cultured from a CF patient during a 3.5 year infection to determine 1) if *B. cenocepacia* LPS is structurally modified during chronic infection and 2) the effect of any modification on the host response to LPS

**Methods**

The LPS was extracted using hot phenol extraction and analysed via SDS-PAGE. The LPS was incubated with CFBE41o-, 16HBE14o- and HL60 cells. The pro-inflammatory cytokines were subsequently analysed by ELISA. The LPS mutant was generated by deletion of BCAL2405, an O-antigen biosynthesis gene using a tri-parental mating method.

**Conclusions**

The O-antigen domain was present in the LPS from the first isolate but absent from the three later isolates. The LPS from the later isolates induced a significantly greater pro-inflammatory response from CFBE41o- cells ( $P < 0.001$ ), 16HBE14o- cells ( $P < 0.05$ ) and HL60 cells ( $P < 0.001$ ) when compared to LPS from the initial isolate. These data suggest that the loss of O-antigen in the later isolates may be responsible for the increased pro-inflammatory response. To verify this, we have successfully deleted the BCAL2405 gene from *B. cenocepacia* K56-2 which yielded LPS without the O-antigen and are currently investigating the pro-inflammatory potential of the mutant strain.

**FEMS-2689**

**Free subjects - 2**

**MIRRI – MICROBIAL RESOURCE RESEARCH INFRASTRUCTURE – DIFFERENT  
WAYS OF COMMUNICATION BETWEEN PROVIDERS AND USERS OF  
CULTURE COLLECTION OF MICROORGANISMS**

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**Background**

Millions of species of microorganisms on the earth, a large amount of them still undiscovered, are the source of biological diversity. Microorganisms are not only indispensable for life; they are basic for many human activities. Microorganisms are an essential part of the day-to-day work not only for scientists, but also for people working in the area of e.g. biotechnology, agriculture, industry, or medicine. Therefore, their economic value is very high. A large percentage of this microbial material is preserved in culture collections (CCs), in which their stability and reproducibility can be protected and through which they are (in most cases) available for other researchers.

**Objectives**

The implementation of the strategy on improved communication between user and provide has to be organised in a coordinated way. By arranging a major part of the CCs pro-active communication through the MIRRI portal the users will get a facilitated access to all requested information.

**Methods**

The main objectives of CCs and microbial resource centres (MRCs) are, besides conserving biodiversity, sharing microbial resources and associated data.

**Conclusions**

MIRRI has prepared strategy for communication between providers, which are Culture Collections with status of Microbiological Resource Centre (MRC) and users of microbiological resources. MRC are public resource centers specialized on microbial

raw material. They are capable of providing for users with culturable organisms such as micro-organisms, plant, animal and human cells, replicable parts of these (genomes, plasmids, viruses, cDNAs), as well as databases containing molecular, physiological and structural information relevant to these collections and related bioinformatics.

**FEMS-2916**

**Free subjects - 2**

## **ELECTROTAXIS AND DUROTAXIS INFLUENCE THE BEHAVIOUR OF SINGLE CELL PSEUDOMONAS AERUGINOSA**

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### **Background**

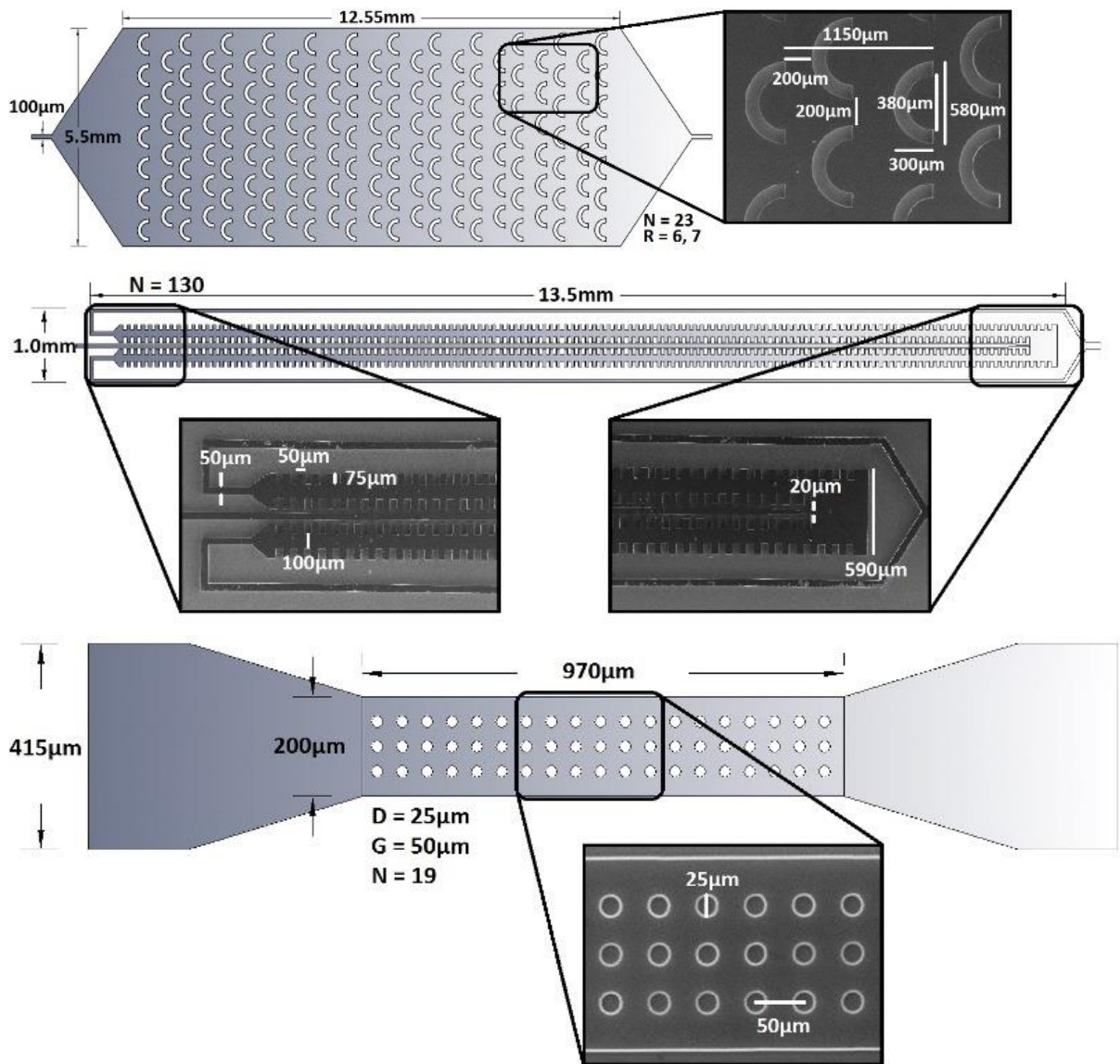
*P. aeruginosa* motility plays a pivotal role in the colonization of surfaces, especially during the formation of antibiotic resistant biofilm structures. Endogenous electrical signals represent an important signaling mechanism that guides cell migration during wound healing, ultimately regulating the direction of cell movement. We report an approach to study the motility of single *P. aeruginosa* cells in microfluidic channels possessing different structural geometries, all with the flexibility of being able to manipulate chemical concentration gradients and electric fields to investigate changes in motility in response to specific stimuli.

### **Objectives**

This study is designed to examine the interplay between surface rigidity, mechanical, and electrical cues to pave the way for improvements in the design of anti-fouling surfaces for biomedical applications and to identify new ways to inhibit bacterial biofilm growth through motility restriction.

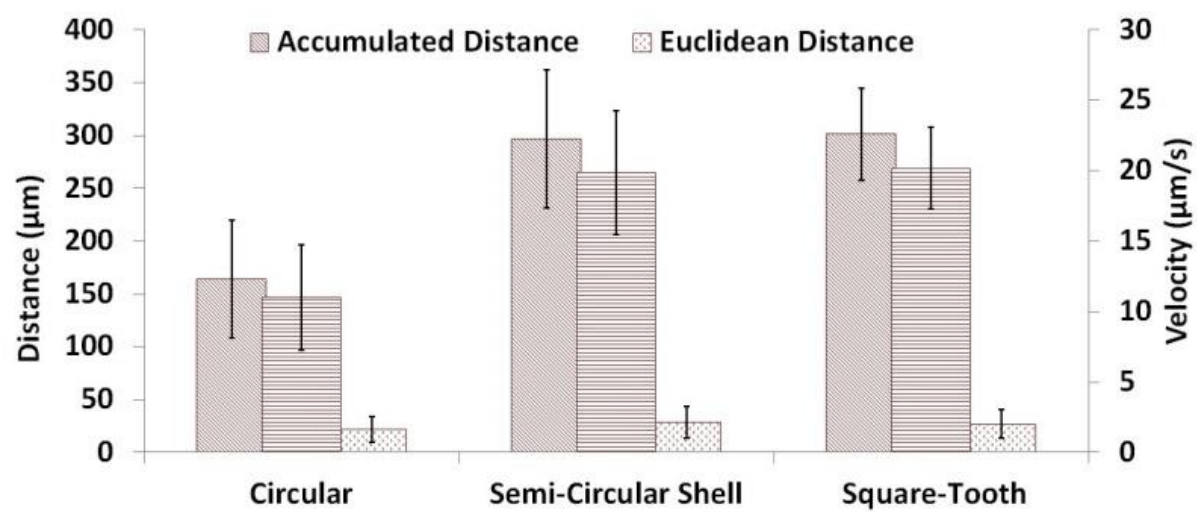
### **Methods**

Three different microfluidic devices with varying geometries (circular, semi-circular shell, and square cut out respectively) were developed using soft-lithography and nanofabrication tools for the analysis of durotaxis and galvanotaxis of single cell *P. aeruginosa*.



## Conclusions

With the use of fabricated microfluidic platforms, we studied the impact of spatial restrictions, rigidity of the substrate medium, and the impact of external electrical fields on *Pseudomonas aeruginosa* motility. Spatial restrictions resulted in a significant reduction in single-cell velocity and the distance travelled, but did not affect the trajectory directness. *Pseudomonas aeruginosa* single cells showed a preference for an environment with increased stiffness, which was independent of the time of exposure to the electric field.



**FEMS-3045**

**Free subjects - 2**

## **MARINE-DERIVED *PENICILLIUM* IN KOREA: DIVERSITY, ENZYME ACTIVITY, AND ANTIFUNGAL PROPERTIES**

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### **Background**

In Korea, approximately 100 *Penicillium* species have been recorded. Many of these species were isolated from soil, and some were found to be associated with post-harvest diseases of plant products. However, the diversity of marine-derived *Penicillium* in Korea is poorly understood relative to terrestrial species.

### **Objectives**

We explored the diversity of marine-derived *Penicillium* in Korea by isolating *Penicillium* species from various marine substrates and identifying them using a multigene phylogenetic approach. We also evaluated the biological activity (extracellular enzyme activity and antifungal activity against the plant pathogens) of the strains.

### **Methods**

The diversity of marine-derived *Penicillium* from Korea was investigated using morphological and multigene phylogenetic approaches (ITS, *BenA*, *RPB2*). We tested for the extracellular enzyme activity of alginase, endoglucanase, and beta-glucosidase, and antifungal activity against two plant pathogens (*Colletotrichum acutatum* and *Fusarium oxysporum*).

### **Conclusions**

A total of 184 strains of 36 *Penicillium* species were isolated, with 27 species being identified. The most common species were *P. polonicum* (19.6 %), *P. rubens* (11.4 %), and *P. chrysogenum* (11.4 %). The diversity of *Penicillium* strains isolated from soil and marine macroorganisms was higher than the diversity of strains isolated from seawater. While many of the isolated strains showed alginase and beta-glucosidase activity, no endoglucanase activity was found. More than half the strains (50.5 %) showed antifungal activity against at least one of the plant pathogens tested. The

relatively high proportion of strains that showed antifungal and enzyme activity demonstrates that marine-derived *Penicillium* have great potential to be used in the production of natural bioactive products for pharmaceutical and/or industrial use.



**FEMS-3036**

**Free subjects - 2**

**SPECIES SPECIFIC-DNA MARKERS FOR CULTURE-INDEPENDENT  
DETECTION AND QUANTIFICATION OF BACILLUS LICHENIFORMIS IN FISH  
GUT**

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**Background**

In fish farming the benefits of probiotics have been usually inferred appraising the effects observed on the host, namely growth, survival and immune response, and not through the direct assessment of probiotics dynamics and role in the host gut microbiota. Currently, there is the urgency to develop accurate tools to assess and weigh up the dynamics of autochthonous bacteria and probiotics in the gut microbiota, in order to better understand the effects of probiotics, prebiotics and synbiotics in aquaculture production.

**Objectives**

This work aimed to identify novel taxa-specific DNA markers for *B. licheniformis*, one of the most used probiotics in aquaculture, and develop culture-independent methods for detection and quantification of these bacteria and understand their modulation role of fish-gut microbiota.

**Methods**

Several putative *B. licheniformis*-specific DNA markers were selected using the

CUPID and Insignia databases. These utilities, combined with comprehensive genomic studies and dedicated experimental validation, allowed the selection of four DNA markers used for detection and quantification of *B. licheniformis* by multiplex PCR and qPCR, respectively, in digesta samples of fish gut samples.

### **Conclusions**

The selected DNA markers were successfully applied for detection and quantification of *B. licheniformis* in fish gut. These results are a contribution for a better understanding of *B. licheniformis* dynamics in gut microbiota of farmed fish in response to different diets, but also during disease outbreaks. This knowledge is an added-value to optimize and implement best practices for aquaculture.

**FEMS-1558**

**Free subjects - 2**

**ICLIKVAL: COMMUNITY RESOURCE FOR CURATING THE VAST WEALTH OF MICROBIOLOGY-RELATED LITERATURE THROUGH THE POWER OF CROWDSOURCING**

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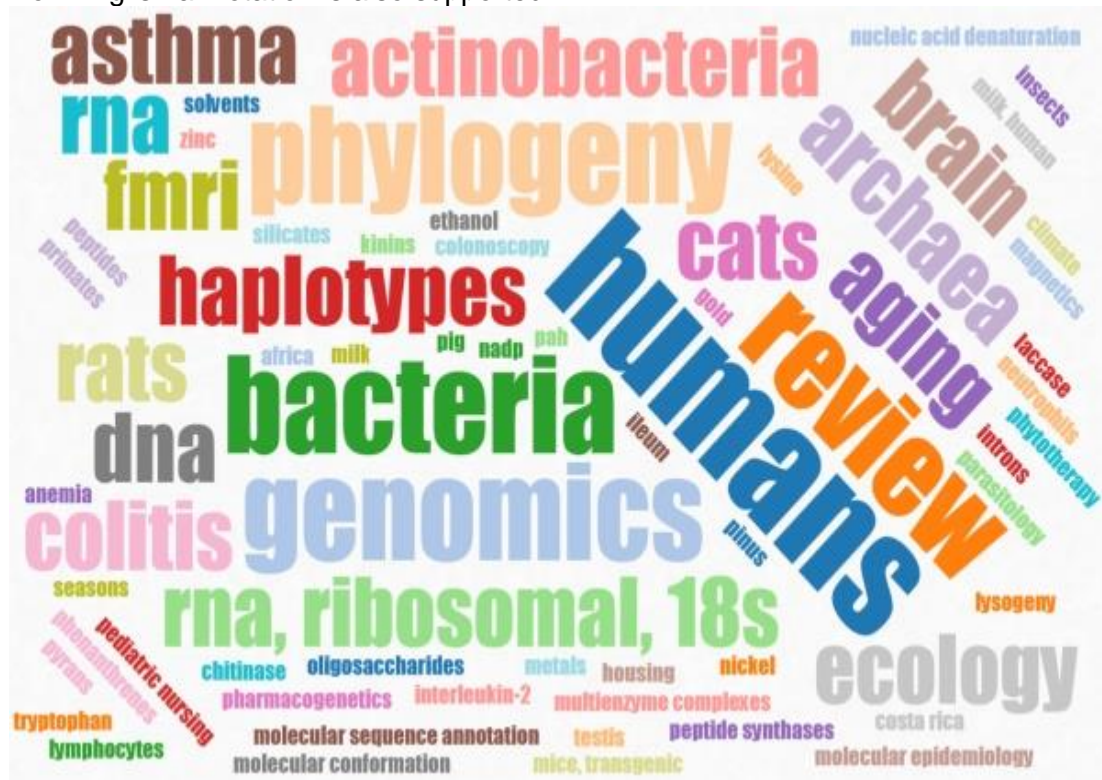
**Background**

There are currently over 24 million citations in PubMed, including many related to microbiology research. Searching this vast resource does not always give desirable or complete results due to several factors. Ideally, every citation should include a complete set of keywords that describe the original article in detail; however, this would require countless hours of manual curation.

**Objectives**

Our goal is to make manual curation 'fun', social, and self-correcting, thus enriching resources like PubMed so that users are able to extract more valuable and relevant results. We developed a web-based open-access tool for manual curation of PubMed articles, and other media types, using a crowdsourcing approach. We encourage the use of ontology terms and support them as auto-suggest keyword terms, but we do not restrict users to these so as not to impose any limitations on the annotation types.

Non-English annotation is also supported.



## Methods

We constructed a cross-browser and platform-independent application using a NoSQL database. Users perform searches, mark articles for review, load PDFs into the viewer, select annotations (values) within the text, and add appropriate keywords (keys). Article-specific comments can be made, key-value pairs can be edited and rated, live chats between users can be conducted, annotations can be added via Twitter, etc.

## Conclusions

The more annotations that accumulate in the database the more reliable the results. We implemented a REST API to make the annotations easily accessible to the research community. We hope this will become the default resource for community-based curation of all online microbiology-related and scientific literature.

**FEMS-3137**

**Free subjects - 2**

**THE RISK OF SERIOUS INFECTIONS WITH THE USE OF RITUXIMAB IN RHEUMATOID ARTHRITIS. RESULTS FROM A META-ANALYSIS OF RANDOMISED CLINICAL TRIALS.**

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**Background**

The association between serious infections (SINFs) and the use of rituximab in patients with non-Hodgkin's lymphoma has been clearly confirmed; but, uncertainty remains about the association of SINFs with rituximab in rheumatoid arthritis (RA).

**Objectives**

Our study, a systematic review and meta-analysis (SR&MA), aimed to provide reliable assessment of the risk of SINFs and rituximab in RA (RTX-RA).

**Methods**

This SR&MA was registered with the PROSPERO database (CRD42014015655) as protocol for a complete evaluation of the safety profile of RTX-RA. The search strategy involved randomised clinical trials using rituximab in RA, published from January 1990 to December 2014 in Medline, EMBASE and Cochrane Library databases. The outcomes evaluated were: the number of adverse events and SINFs reported as outcomes of interest. Odds ratio (OR) analysis, 95% confidence intervals (CI95%) and p values (chi-squared) were calculated; heterogeneity was assessed using the I<sup>2</sup> test. Sub-analysis by doses (500 vs 1000 mg) and type of infection were performed.

Results: Eight publications (3,272 subjects) were selected for review and 5 publications (2,249 subjects) were included in the meta-analysis; the number of events of SINFs in rituximab+methotrexate (RTX-MTX) group vs control (placebo+methotrexate) were 35/1,465 vs 27/784. We found no evidence of association between RTX-MTX and SINFs (OR 0.64; CI95% 0.39-1.04; p=0.74; I<sup>2</sup>=0.000). Similarly, our sub-analysis by RTX doses (500 vs 1000 mg) and type of infection, showed no evidence of association.

## **Conclusions**

Our results suggest no evidence of association between RTX-MTX in RA and SINFs. Further studies are needed to confirm our findings.

**FEMS-1473**

**Free subjects - 2**

**AUDIT OF NOTIFIABLE DISEASE REPORTING IN A GENERAL MEDICINE  
DEPARTMENT OF A DISTRICT GENERAL HOSPITAL IN THE UK**

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**Background**

“The purpose of notification [of infectious diseases] is to enable the prompt investigation, risk assessment and response to cases...that present a significant risk to human health. Notification has the secondary benefit of providing data for use in... epidemiological surveillance...”<sup>1</sup> Doctors in England have a duty to notify diseases as a legislative requirement.

**Objectives**

To establish whether general medical doctors in a hospital in London are appropriately reporting notifiable diseases according to 2010 health protection guidance.

**Methods**

Notifiable diseases managed in adult secondary care were selected: acute meningitis, invasive group A streptococcus, enteric fever, meningococcal septicaemia, malaria.

Adult patients diagnosed with these diseases between May and October 2013 were identified via the microbiology database and clinical coding. The notes were reviewed for documentation of discussion with public health or the notification form, and the time this occurred relative to diagnosis/clinical suspicion.

**Conclusions**

24 cases of notifiable diseases were identified and of these 17 notes were located (7 malaria, 9 viral meningitis, 1 *Salmonella typhi*). There was 1 documented notification (6% of 17 notes), with no identifiable date.

Possible causes of the low notification rate include: poor awareness among doctors that it is their responsibility, lack of knowledge which diseases are notifiable, uncertainty which clinician/clinical team has responsibility for notification, and difficulty in locating forms on the hospital intranet.

Measures to improve notification include implementation of electronic notification reminders with results, staff education and more easily accessible information/notification form on intranet.

**References:** 1. Department of Health. Health Protection England Legislation (England) Guidance 2010



**FEMS-1649**

**Bacterial cell biology**

**CHARACTERIZATION OF THE LIPOPOLYSACCHARIDE TRANSPORT MACHINE IN ESCHERICHIA COLI: IN SEARCH OF A FUNCTION FOR THE ELUSIVE COMPONENT LPTC**

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**Background**

The lipopolysaccharide (LPS) transport (Lpt) system is responsible for transferring LPS from the periplasmic surface of the inner membrane (IM) to the outer leaflet of the outer membrane (OM), where it plays a crucial role in OM selective permeability. In *E. coli* seven essential proteins form the Lpt trans-envelope complex: LptBFG form the IM ABC transporter; LptDE form the OM translocon for final LPS delivery; LptC, an IM-anchored protein with a periplasmic domain, interacts with the IM ABC transporter, the periplasmic protein LptA, and LPS<sup>(1)</sup>. Although essential, LptC can tolerate several mutations and its role in LPS transport is unclear.

**Objectives**

To clarify the functional role of LptC in the Lpt machine.

**Methods**

By plasmid shuffling we obtained viable mutants missing *lptC*; genome sequencing of  $\Delta$ *lptC* mutants revealed single amino acid substitutions at a unique position (\*) in the IM component *lptF*; in complementation tests, *lptF*<sup>\*</sup> mutants suppress lethality of both  $\Delta$ *lptC* and *lptC* conditional expression mutants.

**Conclusions**

Our data show that a specific mutation in LptF can compensate the lack of LptC and suggest that LptC may serve as a chaperon of the Lpt machine assembly and/or activity rather than an essential structural component. Studies are in progress to understand the structure and function of the six-protein Lpt<sup>\*</sup> system.

<sup>(1)</sup> Sperandeo *et al.* (2014) The outer membrane of Gram-negative bacteria: lipopolysaccharide biogenesis and transport. In: Remaut, H. and Fonzes, R. (Eds) Bacterial membranes: structural and molecular biology. pp. 55-90. Horizon Scientific Press.

**FEMS-0766**

**Bacterial cell biology**

## **A NOVEL SCENARIO OF OPRF REGULATION IN PSEUDOMONAS AERUGINOSA**

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### **Background**

OprF, the major porin of *Pseudomonas aeruginosa*, has an immunogenic potential and a large set of information regarding its functions, including its role in the outer membrane structure, interaction with lipopolysaccharide and association with virulence and quorum sensing. In the prototype strain PAO1, transcription of *oprF* was reported to be dependent on sigma 70 and the alternative ECF sigma factor SigX.

### **Objectives**

As we have shown that SigX overexpression in PA14 leads to a growth defect, we assessed *oprF* transcription regulation in order to verify if this effect is due to OprF overexpression and to characterize *oprF* regulation in PA14.

### **Methods**

*oprF* promoter activity was determined via *-lacZ* reporter fusions integrated in the chromosome or by relative *oprF* mRNA levels (qRT-PCR) in wild-type PA14, *sigX* null mutant or in PA14 overexpressing *sigX* from an inducible promoter in a multicopy plasmid (ALB04 strain). *oprF* and *sigX* promoters activity was also assessed in the PA14/*rpoS* mutant by  $\beta$ -galactosidase assays.

### **Conclusions**

Surprisingly, we found that all *oprF-lacZ* promoters activity decreased in ALB04 and the amount of OprF did not increase in a proteomic analysis. The *sigX* null mutant has wild-type levels of *oprF* mRNA, contrasting to other SigX-induced genes. We have also found that transcription of *oprF* occurs mainly via the region containing the putative sigma 70-dependent promoter. Although *oprF* transcription regulation is independent of SigX in PA14, both *oprF* and *sigX* transcription is dependent on the general stress sigma factor RpoS. The data presented here discloses a novel

scenario of *oprF* regulation in *P. aeruginosa*.

**FEMS-1268**

**Bacterial cell biology**

**TRIGGER ENZYME PEPA FROM *E. COLI*, A TRANSCRIPTIONAL REPRESSOR THAT GENERATES POSITIVE SUPERCOILING**

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**Background**

Hexameric *E. coli* Leucine-aminopeptidase A (PepA) is a trigger enzyme endowed with catalytic activity and DNA-binding properties prominent in the resolution of ColE1 and pSC101 plasmid multimers and transcriptional regulation of the *carAB* operon, encoding the unique carbamoylphosphate synthase of *E. coli*. Previous studies by DNase I footprinting and atomic force microscopy (AFM) had both indicated a pronounced deformation of the *carAB* control region upon PepA binding, suggestive of DNA wrapping. On the basis of this observation and previous work, PepA was believed to play a major, although merely architectural role in the formation of the synaptic complexes involved in site-specific DNA recombination and in the elaboration of a higher order regulatory protein-*carP1* DNA complex.

**Objectives**

Here we further investigate the molecular mechanism of PepA-mediated transcriptional regulation of the *carP1* promoter.

**Methods**

To this aim we use single-round *in vitro* transcription and DNA topology assays.

**Conclusions**

The *in vitro* transcription assays performed with supercoiled template and purified components demonstrate that PepA is a repressor in its own right, which specifically inhibits transcription initiation at *carP1*. Furthermore, DNA topology studies performed on artificial DNA mini-circles by means of various topoisomerases with different substrate specificities demonstrate that PepA binding induces positive supercoiling. Such topological changes may serve as a regulatory mechanism that allows swifter response and multi-layered control of promoter activity in concert with other regulatory components known to be involved in the control of *carP1*.

**FEMS-1148**

**Bacterial cell biology**

**OFF THE WALL: FROM FILAMENTOUS GROWTH TO PRIMORDIAL CELLS AND BACK AGAIN**

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**Background**

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**Objectives**

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**Methods**

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**Conclusions**

Streptomyces are filamentous bacteria that grow by apical tip extension. This process is orchestrated by the tropomyosin-like protein DivIVA, which is present at hyphal tips. DivIVA interacts with various proteins, among which the cellulose synthase-like protein CslA. This protein synthesizes a  $\beta$ -(1,4)-glycan, which is thought to protect growing apices that are continuously being remodeled. To obtain further insight in the role of DivIVA and CslA in polar growth and morphogenesis, we have recently generated so-called *Streptomyces* L-forms that can grow without peptidoglycan. As a consequence, such cells are round and lack any obvious form of polarity. L-form cells have recently been suggested to resemble primordial cell, based on the observation that their growth and proliferation do not require the canonical cytoskeletal or cell division proteins. Instead, their proliferation can merely be explained by physical processes. However, our work on *Streptomyces* L-forms suggests that these cells require glycans, such as those formed by CslA, for their growth. Such glycans might have served for protection of early life forms, before the modern cell wall was invented. We have recently isolated an L-form mutant strain, which readily switches back and forth between mycelial and L-form growth. This mutant with the capability to re-synthesize peptidoglycan is crucial to understand which genes play an essential role in proliferation of L-forms, but also to unravel the mechanism underlying filamentous growth.

**FEMS-1921**

**Bacterial cell biology**

**THE ESCHERICHIA COLI MEMBRANE PROTEIN INSERTASE YIDC ASSISTS IN THE BIOGENESIS OF PENICILLIN BINDING PROTEINS**

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**Background**

Membrane proteins need to be properly inserted and folded in the membrane in order to perform a range of activities that are essential for the survival of bacteria. The Sec translocon and the YidC insertase are responsible for the insertion of the majority of proteins into the cytoplasmic membrane. YidC can act in combination with the Sec translocon in the insertion and folding of membrane proteins. However, YidC also functions as an insertase independently of the Sec translocon for so-called YidC-only substrates. In addition, YidC can act as a foldase and promote the proper assembly of membrane protein complexes.

**Objectives**

Here, we investigate the effect of *Escherichia coli* YidC depletion on the assembly of Penicillin Binding Proteins (PBPs), that are involved in cell wall synthesis.

**Methods**

Active PBPs were assessed by substrate binding and total protein amount was determined by immunoblotting.

**Conclusions**

YidC depletion hardly affects the total amount of the specific cell division PBP3 (FtsI) in the membrane, but the amount of active PBP3 is strongly reduced. Similar reductions in the amounts of active PBP2 and PBP4 were observed, while the levels of active PBP1A/1B and PBP5 were essentially similar. PBP1B and PBP3 disappeared from higher Mw bands upon YidC depletion, indicating that YidC might play a role in PBP complex formation. Taken together, our results suggest that the foldase activity of YidC can extend to the periplasmic domains of membrane proteins.

**FEMS-2367**

**Bacterial cell biology**

## **INTERPLAY BETWEEN UVRD HELICASE AND RECJ EXONUCLEASE IN NEISSERIA GONORRHOEAE**

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### **Background**

*Neisseria gonorrhoeae* is an obligate human pathogen causing gonorrhea. It mainly employs homologous recombination to repair double stranded breaks in DNA. The involvement of mismatch repair helicase, UvrD in homologous recombination is debatable till date. Evidences have implied that it could be an anti-recombinase or promote RecFOR pathway. In this study, a detailed biochemical characterization of UvrD from *N. gonorrhoeae* and its interaction with RecJ exonuclease (NgoRecJ) which is involved in resection of DNA ends during RecFOR pathway of recombination has been carried out.

### **Objectives**

1. To biochemically characterize UvrD from *Neisseria gonorrhoeae* (NgoUvrD).
2. To study the physical interaction between NgoUvrD and NgoRecJ exonuclease.
3. To elucidate the functional role of this interaction, if any.

### **Methods**

Both the proteins were purified by Ni<sup>2+</sup>-NTA followed by Heparin-Sepharose chromatography. The unwinding and exonuclease activities were monitored using gel based assays. ATPase assays were carried out using TLC. Protein-protein interaction studies were done using Far-western analysis.

### **Conclusions**

Biochemical analysis suggested that NgoUvrD behaves as a dimer in solution, exhibits 3'-5' polarity on ssDNA and can unwind blunt end duplex DNA as well as different recombination intermediates such as overhang containing DNA and Holliday junction in ATP dependent manner. It was observed that NgoRecJ and NgoUvrD

physically interact with each other. Interestingly, the ATPase activity of NgoUvrD shows 5- fold stimulation in presence of NgoRecJ. This observation for the first time shows that UvrD in complex with RecJ exonuclease might be involved in the initial step of RecFOR pathway, thereby promoting recombination.



**FEMS-1238**

**Bacterial cell biology**

## **A NEW PROGRAMMED CELL DEATH PATHWAY IN ESCHERICHIA COLI**

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### **Background**

Programmed cell death (PCD) in bacteria has long been controversial due to the belief that only multicellular organisms would benefit from this kind of altruistic behavior. However, over the past decade, compelling experimental evidence has established a consensus that such pathways exist in bacteria.

### **Objectives**

Recently, we discovered that expression of a mutant isoform of an essential GTPase, ObgE, causes rapid cell death in *Escherichia coli*. The physiological changes that occur upon expression of this mutant protein (ObgE\*) and the genetic basis of this cell death pathway were investigated.

### **Methods**

Besides a clear loss of viability, fluorescence microscopy and FACS analysis revealed that a large array of apoptotic markers – including chromosome condensation, DNA fragmentation, loss of membrane potential and exposure of phosphatidylserine on the cell surface – can be found upon expression of ObgE\*, indicating that ObgE\* triggers a PCD pathway. Previous reports of bacterial PCD attribute essential roles to either RecA and its function in the SOS response or to the toxic effect of the *mazEF* TA module. However, by analysing viability and physiological changes upon ObgE\* expression in *recA* and *mazEF* mutant strains, all currently known bacterial PCD mechanisms could be excluded.

### **Conclusions**

ObgE\* triggers a PCD pathway in *E. coli* that differs fundamentally from all other previously described bacterial PCD pathways. Further research into the genetic and molecular basis of this new PCD mechanism may prove useful in unraveling the function and mechanisms of genetically encoded cell death pathways in bacteria.

**FEMS-1597**

**Bacterial cell biology**

## **MUTATIONAL ANALYSIS OF LPTA, AN ESSENTIAL LPS-TRANSPORT PROTEIN IN ESCHERICHIA COLI**

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### **Background**

Lipopolysaccharide (LPS) is the major outer leaflet constituent of the Gram-negative bacteria outer membrane (OM). In *E. coli* LptA protein is a periplasmic component of the LPS transporter (Lpt) made of seven components (LptABCDEFG), which ferries LPS from the inner membrane (IM) to OM. LptA interacts with LptC and chaperons LPS through the periplasm. The crystal structure of LptA has been solved and some residues involved in binding LPS and other Lpt proteins have been identified.

### **Objectives**

1. To characterize LptA structure-function.
2. To identify interactions between genes/proteins implicated in OM functionality.

### **Methods**

1. We generated by site-directed mutagenesis *lptA41*, a quadruple mutant in four conserved amino acids potentially involved in LPS or LptC binding; the mutant exhibited increased sensitivity to hydrophobic compounds (Shc phenotype). This suggests that *lptA41* impairs LPS transport thus leading to OM permeability defects.
2. We selected suppressors of *lptA41* Shc phenotype and sequenced the genomes of two mutants (Sup102 and Sup103) to identify the suppressing mutations.

### **Conclusions**

Sup102 harbors a small in-frame deletion in *vacJ*, which codes for a putative OM lipoprotein involved in maintaining OM asymmetry. Sup103 harbors an additional amino-acid substitution (M112I) in LptA41 (*lptA42* allele) and a nonsense mutation in *mdoH*, a gene encoding a glycosyltransferase involved in periplasmic membrane-derived oligosaccharides synthesis.

These results reveal two different strategies adopted by the cell to overcome OM defects caused by defective LPS transport.

**FEMS-2754**

**Bacterial cell biology**

**INVESTIGATIONS ON THE BIOLOGICAL SIGNIFICANCE OF BURKHOLDERIA CENOCEPACIA J2315 HFQ2 PROTEIN ABILITY TO BIND DNA**

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**Background**

Accumulating evidence has shown that Hfq is a RNA chaperone involved in post-transcriptional regulation, mediating the interaction of small RNAs with their target mRNAs. However, besides its regulatory role in the translation of bacterial mRNA, in *Escherichia coli*, Hfq is also a DNA-binding protein and is associated with nucleoid DNA. Bcc bacteria are among the few prokaryotes that encode in their genomes two distinct and functional Hfq-like proteins, the 79 aa Hfq and the 188 aa Hfq2. Both proteins bind RNA, however bioinformatics predictions suggested that Hfq2 is able to bind DNA by means of its C-terminal domain.

**Objectives**

The present study aims to unveil the biological significance of Hfq2 C-terminus predicted ability to bind to DNA.

**Methods**

A DNA binding domain was bioinformatically predicted for Hfq2 and DNA binding assays were performed using purified His-tagged Hfq or Hfq2 and the *araC* promoter of pMLBAD plasmid. Electrophoretic Mobility Shift Assays indicates that Hfq2 protein, but not Hfq, is able to bind this DNA fragment. Hfq2 derivatives with complete, partial and absent C-terminus extension were produced to confirm if this region confers the ability to bind DNA. To identify the DNA sequences to which Hfq2 specifically binds, a ChIP-seq strategy is currently being performed, using *B. cenocepacia* J2315 fragmented total DNA.

**Conclusions**

Contrasting with Hfq, a putative DNA binding domain was identified in Hfq2 and our results showed that this protein is able to bind DNA. Ongoing work is being performed to identify the DNA sequences and the specificity of DNA-Hfq2 interactions.



**FEMS-1260**

**Bacterial cell biology**

## **KNOCKOUT OF MINC LEADS TO BRANCHING IN XANTHOMONAS CITRI**

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### **Background**

*Xanthomonas citri* (Xac) is the causal agent of citrus-canker, a disease that affects citrus plants worldwide, leading to low productivity, culminating in considerable economic losses to orange growers. There is no treatment for citrus canker, and eradication of plants is the only reliable method to prevent the spread of Xac to areas considered free of the disease. We reported on the use of new cell-division inhibitors as an alternative to prevent Xac growth, and plant colonization (Dantas *et al.* *JBacteriol* **195**:85). To further extend the characterization of cell division in Xac, we started the investigation of the *minC* gene.

### **Objectives**

To study *minC* encoded by Xac and its function in site division selection at cell division

### **Methods**

The *minC* gene was deleted using allele exchange. The knocked-out strain was complemented with a replicon containing *minC* under the control of the arabinose promoter. Pathogenicity tests were carried out by infiltration of sweet orange (*Citrus sinensis* (L.) Osbeck). Cell morphology was evaluated using DIC microscopy

### **Conclusions**

Xac deleted for *minC* exhibited the classic delta-*minC* phenotypes: production of minicells, cell filamentation, and asymmetric/misplaced division constrictions along the rods. Surprisingly, Xac delta-*minC* produced branching, a phenotype observed for *Escherichia coli* deleted for PBP5 and other low-molecular-weight penicillin-binding proteins. Complementation of delta-*minC* with *minC* supplied *in trans* restituted the wild-type division phenotype. Irrespective of the phenotype, the delta-*minC* strain was competent to colonize the host citrus and produce disease symptoms. Altogether, data shows that *minC* is essential for normal cell division in Xac.

**FEMS-1003**

**Bacterial cell biology**

**CSRA PARTICIPATES TO ENVELOPE HOMEOSTASIS IN ESCHERICHIA COLI**

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**Background**

The CsrA protein (Carbon Storage Regulator A) is a global post-transcriptional regulator controlling carbon fluxes and group behaviors in bacteria. Our preliminary SEM analyses revealed that an *E. coli* mutant deleted for the *csrA* gene displays a strongly altered morphology. Cells are of small size as compared to the wild-type cells and present an irregular surface and blebs.

**Objectives**

Cell envelope is the first line target for physicochemical injuries arising from the environment. This complex structure also supports fundamental processes such as energy production and nutrient acquisition. To preserve envelope homeostasis bacteria have evolved intricate stress response pathways. The objective is to evaluate whether envelope stress responses are induced in  $\Delta csrA$  mutant and if it is the case, what are the underlying molecular mechanisms.

**Methods**

Expression of effectors belonging to the 5 well-described envelope stress response pathways (*bae*, *psp*, *cpx*, *sigmaE*, *rsc*) were analyzed by RT-qPCR. Envelope integrity was evaluated by measuring sensitivity to specific compounds.

**Conclusions**

We have shown that in the  $\Delta csrA$  mutant, the Psp and Rcs pathways are constitutively induced. Rcs pathway monitors outer membrane integrity while Psp that of the inner membrane. This mutant also shows a high sensitivity to deoxycholate and vancomycin, indicating an increased permeability of the outer membrane. In addition, the  $\Delta csrA$  mutant accumulates high ROS concentration, which might participate to *psp* activation. ROS accumulation might thus represent the link between metabolism and envelope defects. This hypothesis is currently under investigation.

**FEMS-1890**  
**Bacterial cell biology**

**IDENTIFICATION OF THE MULTI-COMPONENTS DNA UPTAKE SYSTEM OF NATURALLY TRANSFORMABLE LEGIONELLA PNEUMOPHILA USING TRANSCRIPTOMIC ANALYSIS.**

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**Background**

Natural transformation is a major mechanism of horizontal gene transfer that is already well-studied in historical Gram-positive models whereas knowledge on Gram-negative bacteria remains sparse. *Legionella pneumophila* is a Gram-negative bacterium that develops, under specific growth conditions or stress, competence for natural transformation, a genetically programmed state allowing bacteria to take up free exogenous DNA and integrate it in their genome. The *L. pneumophila* genome shows a large repertoire of virulence-associated eukaryotic-like genes. Natural transformation offers a plausible route for acquisition of foreign genes contributing to the emergence of this pulmonary pathogen.

**Objectives**

The DNA uptake system allowing *L. pneumophila* to take up DNA and recombine it into its chromosome remains unknown. The aim of our study was to identify this system and determine its contribution to the infectious process.

**Methods**

We used a transcriptomic analysis to identify genes up-regulated in an hypercompetent mutant of *L. pneumophila*. We performed a systematic targeted mutagenesis of these up-regulated genes to test their involvement in transformability and DNA uptake. We used immunofluorescence microscopy to visualize components of the DNA uptake system.

**Conclusions**



Our work allowed us to identify the main components of the *Legionella* DNA uptake system which involves a type IV transformation pilus dedicated to natural transformation. We propose a model of the DNA uptake system that unexpectedly relies on the actin-like protein MreB. These progresses pave the way for a more detailed analysis of the DNA uptake system dynamics and more broadly for a better understanding of Gram-negative bacteria transformation systems.

**FEMS-1544**

**Bacterial cell biology**

## **THE MOLECULAR BASIS OF REGULATION OF TNR DNA-BINDING ACTIVITY IN BACILLUS SUBTILIS**

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### **Background**

TnrA is a master transcriptional regulator of nitrogen metabolism in *Bacillus subtilis*. Its activity was suggested to be regulated by binding to the feedback-inhibited glutamine synthetase (GS). Recently we showed that TnrA also binds to the PII-like GlnK protein.

### **Objectives**

Understanding of TnrA activity regulation via interaction with GlnK or GS

### **Methods**

*In vivo* cross-linking, Surface Plasmon Resonance, ITC

### **Conclusions**

We found that *in vivo* TnrA binds both proteins, but in contrast to the suggested mechanism, TnrA binds GS also under nitrogen-poor conditions. SPR analysis demonstrated that GS in the absence of feed-back inhibitors efficiently binds TnrA, but 1 mM glutamine strengthens the binding 2-fold. By contrast, ATP strongly repressed TnrA-GS interaction, whereas 1 mM glutamine completely neutralized this negative effect. In presence of L-methionine sulfoximine (MSX), a transition-state analogue of GS, GS–TnrA interaction was also stimulated. However, in presence of MSX and ATP, where MSX is phosphorylated and irreversibly fixes GS in transition state, GS binding to TnrA was completely abolished. Isothermal titration calorimetry revealed competitive binding of glutamine and ATP to GS. Therefore, we suggest two conformations of GS for TnrA interaction: (1) a TnrA-binding “N-state”, stabilized by glutamine or its analogue MSX and (2), a non-binding “A-state”, which reflects the conformation of the enzyme in the catalytic transition state or bound with ATP in

absence of glutamine. We propose that GS changes between the “N-state” and “A-state”. In vivo, this transition depends on the intracellular glutamine:ATP ratio and thus regulates the amount of active TnrA.

**FEMS-3056**

**Bacterial cell biology**

**A VIBRIO VUNIFICUS ORTHOLOG OF ESCHERICHIA COLI RRAA  
CONTRIBUTES TO DIFFERENTIAL RNASE E-MEDIATED MRNA DECAY**

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**Background**

Endoribonuclease E (RNase E) plays an important role in the degradation and processing of RNAs in *Escherichia coli*. Expression levels and enzymatic activity of RNase E are tightly controlled by self-cleavage of *rne* mRNA and regulators of RNase activity, RraA and RraB. The marine pathogenic bacterium *Vibrio vulnificus* also contains homologs of RNase E and RraA, designated as RNase EV, RraAV1, and RraAV2.

**Objectives**

In this study, we show that RraAV1 actively inhibits the enzymatic activity of RNase EV on a subgroup of substrate RNAs.

**Methods**

Notably, RNase EV cleavage on *rne* mRNA encoding RNase EV was greatly affected by RraAV1 *in vitro* and *in vivo*, contributing to autoregulation of both expression and activity of RNase EV.

**Conclusions**

Our findings suggest that RraAV1 plays an active role in differential RNase E-mediated mRNA decay.

**FEMS-1346**

**Bacterial cell biology**

**THE STUDY OF BIOGENESIS FEATURES OF OUTER MEMBRANE VESICLES  
PRODUCED BY *LYSOBACTER* SP. XL1**

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**Background**

Gram-negative bacteria produce outer membrane vesicles (OMVs) containing biologically active proteins. Despite of extensive information about OMVs biogenesis available, this process is still not enough clear. Gram-negative bacterium *Lysobacter* sp. XL1 forms OMVs containing bacteriolytic endopeptidase L5, – one of five bacteriolytic enzymes secreted by this microorganism. Knowledge about OMVs biogenesis by bacteria of *Lysobacter* genus is rather restricted.

**Objectives**

The objective of our work was the study of factors causing biogenesis of OMVs produced by *Lysobacter* sp. XL1.

**Methods**

Methods were used: fractionation of OMVs using sucrose density gradient centrifugation, electronic microscopy including immunocytochemistry with the protein A-gold, SDS-PAGE electrophoresis, Western blotting assay, protein and 2-keto-3-deoxyoctonate assays, thin-layer chromatography, determination of lytic OMVs action.

**Conclusions**

Enzyme L5 was found to be localized inside of the lightest OMVs fraction which differed in protein composition from other fractions. The L5 protein was revealed at certain loci of bacterial outer membrane (OM). OMVs were produced from these loci. Thereby, secreting enzyme L5 took part in biogenesis of OMVs. OM was shown to contain cardiolipin, phosphatidylethanolamine, phosphatidylglycerol. OMVs contained the only one major phospholipid, – cardiolipin. Thus, OMVs of *Lysobacter* sp. XL1

were formed from loci enriched with cardiolipin. It has been offered the model of vesicles biogenesis produced by *Lysobacter* sp. XL1. Besides, OMVs containing protein L5 were shown to lyse broad range of opportunistic and pathogenic bacteria including multidrug-resistant strains. The data obtained are the basis for development of artificial vesicular structures – liposomes – containing bacteriolytic enzymes of *Lysobacter* sp. XL1 as a new effective antimicrobial preparation.

**FEMS-1672**

**Bacterial cell biology**

## **HOW TO DEAL WITH COPPER? CAULOBACTER CRESCENTUS' POINT OF VIEW.**

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### **Background**

Despite its essential role as a trace element, copper becomes toxic at higher concentrations, prompting bacteria to develop a tight regulation of copper homeostasis in order to survive.

### **Objectives**

The alphaproteobacterium *Caulobacter crescentus* lives in poor and contaminated environments and gives rise to two distinct cell types upon cell division: a motile swarmer cell and a sessile stalked cell. The swarmer cell is thought to seek optimal environments in order to differentiate into a stalked cell, which will in turn replicate its DNA and divide. This dimorphic cell cycle likely provides a better adaptation to various stresses such as an increase of copper concentration.

### **Methods**

We found that the swarmer and the stalked cells respond differently to a toxic copper concentration. Copper slows down swarmer to stalked cell transition and the further initiation of DNA replication, whereas copper-treated stalked cells undergoes a normal DNA replication. Accordingly, copper concentration in swarmer cells is 3 fold higher than in the stalked cells. We showed that copper homeostasis in the stalked cells is ensured by the conserved PcoAB<sub>Cc</sub> system, which is also able to sustain copper detoxification in the swarmer cells when constitutively expressed in trans, thereby restoring a proper swarmer to stalked cell transition.

### **Conclusions**

Both *C. crescentus* cell types regulate copper homeostasis differently in order to progress throughout the cell cycle.

**FEMS-3057**

**Bacterial cell biology**

**AN RNASE G-MEDIATED PATHWAY FOR PATHOGENICITY IN SALMONELLA  
TYPHIMURIUM**

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**Background**

Endoribonuclease G (RNase G) is involved in rRNA processing and degradation of a subgroup of mRNAs in *Escherichia coli*. However, little is known about physiological role of this enzyme.

**Objectives**

We observed RNase G expression-dependent alterations in the survival rate of *Salmonella* Typhimurium cells in both macrophages and mice. These alterations were strongly associated with expression levels of *rbs* mRNA encoding ribose transport system, which was identified as a substrate of RNase G.

**Methods**

Analysis of polycistronic *rbs* mRNA identified two RNase G cleavage sites in the 5'-untranslated region of *rbsA*. The induced *rbs* expression during macrophage infection coincided with decreased rates of bacterial infection and survival.

**Conclusions**

Our study shows that RNase G mediates a previously uncharacterized pathway that involves ribose transport system as a key factor for the survival and virulence of *S. Typhimurium* in host cells.



**FEMS-2049**

**Bacterial cell biology**

## **IDENTIFICATION AND CHARACTERIZATION OF A CORYNEBACTERIUM GLUTAMICUM MUTANT IMPAIRED IN THE BIOSYNTHESIS OF PEPTIDOGLYCAN**

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### **Background**

Bacteria belonging to *Corynebacteriales*, an order of actinomycetes group that includes corynebacteria, mycobacteria, nocardia and rhodococci possess an atypic, complex multilayered envelope. This envelope is composed of a heteropolymer of peptidoglycan (PG) and arabinogalactan (AG) covalently associated to an outer membrane mostly composed of mycolic acids. We explored the biogenesis of *Corynebacteriales* cell envelope by using a genome-wide transposon mutagenesis approach targeting *Corynebacterium glutamicum*. For this purpose, we developed an effective immunological screen that allows us to rapidly identify bacteria exhibiting an altered cell envelope.

### **Objectives**

We wanted to identify missing actors of the *Corynebacteriales* envelope biosynthesis pathways. One interesting mutant of our library was chosen and characterized in this study.

### **Methods**

We identified the gene inactivated by the transposon and constructed a strain in which this gene was deleted. We performed analyses of the main cell wall compounds of this mutant strain, i.e. PG, AG and mycolic acids. A physiological study was also conducted and in particular a screen for the sensitivity of this mutant to various antibiotics.

### **Conclusions**

Our analyses revealed that this mutant was impaired in the PG biosynthesis pathway and more specifically in a modification of this polymer that also occurs in mycobacteria species and in other *Corynebacterium* pathogens species such as *Corynebacterium jeikeium*. Lack of this modification led to morphologically-altered

cells with high susceptibility to lysozyme and to antibiotics of the  $\beta$ -lactam family.

**FEMS-1865**

**Bacterial cell biology**

**TOWARDS COMPREHENSION OF THE ROLE OF KEY GENES DURING  
LACTOBACILLUS CASEI ESTABLISHMENT IN THE GUT.**

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**Background**

Beneficial interactions between gut microbiota and the host have been revealed but little is known at the molecular level. To identify bacterial factors that account for these symbiotic interactions, we recently carried out reverse genetics of *Lactobacillus casei* which allowed identification of 47 key genes for *L. casei* establishment in the gut (Licandro-Seraut et al. 2014. PNAS).

**Objectives**

Now, we aim to decipher the functions encoded by these key genes in the gut context.

**Methods**

Some of the *L. casei* mutants have been investigated individually with regard to their morphology, metabolism, gene regulation...

**Conclusions**

A mutant for the predicted cysteine synthase was investigated to demonstrate the metabolic role of the cysteine synthase. Also, it was less resistant than the wild type to several stresses. Three mutants for three genes in an operon encoding a two-component system and a penicillin-binding protein were characterized. Analysis of primary data suggests that these genes are involved in the cell surface modulation and particularly in the regulation of peptidoglycan hydrolases which are required for the bacteria protection in the intestinal environment.

**FEMS-2808**

**Bacterial cell biology**

**Role of PprM in catalase gene expression of the radiation-resistant bacterium, *Deinococcus radiodurans***

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Background: *Deinococcus radiodurans* (*D. radiodurans*) is a poly-extremophilic organism, capable of tolerating a wide variety of different stresses such as gamma/UV radiation, desiccation, and oxidative stress. It is known that PprI (DR0167) is a global regulator, which is essential for its extreme resistance, and cold shock protein homologue PprM (DR0907) is presumably controlled by PprI. PprI mutation decreases the catalase activity of *D. radiodurans*, but, the effect of PprM on catalase has not been studied.

Objectives: We investigated the role of PprM, which is involved PprI-mediated signal transduction, in catalase gene expression.

Methods: First, we performed an in-gel catalase activity assay to observe activity of three catalases (DR1998, DRA0146, and DRA0259) independently in wild type, pprM, pprI, and pprI/pprM double mutant strains. We also constructed catalase mutants of *D. radiodurans* to identify the catalase that is affected by PprM and purified catalases to check the activity in vitro. The mRNA and protein levels of catalase genes were monitored by using real-time PCR and western blot assays in various mutant strains.

Conclusions: Among three catalases, we confirmed that DRA0146 does not have catalase activity. Deletion of pprM decreased catalase activity and protein level of DR1998, but not its mRNA level. We could not observe an additional reduction of the DR1998 protein in the pprI/pprM double mutants compared to pprI or pprM single mutants. These suggest that PprI can affect DR1998 via PprM, and PprM may exert its effect on DR1998 at the post-transcriptional level.

**FEMS-2317**

**Bacterial cell biology**

**PHYSIOLOGICAL GLUCOSE CONCENTRATIONS TRIGGER LPS PRODUCTION AND VIRULENCE IN A ACINETOBACTER BAUMANNII.**

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**Background**

*Acinetobacter baumannii* is an emerging pathogen involved in severe infections and outbreaks mainly occurring in clinical settings. Multi-drug resistance and ability to form biofilm could play a role in its persistence in hospital settings.

The present study analyzes an *A. baumannii* clinical strain, belonging to the SMAL clone, isolated in an Italian hospital.

**Objectives**

The aim of this work is to study the effect of glucose, at a concentration similar to human blood, on production of biofilm determinants in *A. baumannii*.

**Methods**

Genome sequence and transcriptome analysis were performed by using deep sequencing and RNA-seq techniques (Illumina).

Transcription of selected genes were measured by using transcriptional fusions with the reporter gene *lacZ*.

Exopolysaccharide (EPS) production was analysed by EDTA extraction and phenol-sulfuric acid quantification.

Lipopolysaccharide (LPS) production was measured using tricine SDS-PAGE.

Cytokine production was tested by ELISA analysis.

**Conclusions**

Genome sequence of the analysed strain showed identity with the sequence type 78, epidemic in several Mediterranean countries.

Growth in the presence of glucose showed higher EPS production; consistently, transcriptome analysis suggested a deep modification of gene expression in presence of glucose. In particular, several genes involved in EPS and LPS biosynthesis and modification were up-regulated in presence of glucose. Remarkably, *A. baumannii* grown in presence of glucose appears to release LPS, which in turn stimulates cytokine production in macrophages.

Our results suggest that glucose at physiological concentrations might act as a trigger for LPS production and virulence in *A. baumannii*.

**FEMS-1759**

**Bacterial cell biology**

## **ACTION OF MOLECULAR CHAPERONES DEDICATED TO THE MATURATION OF REDUCTIVE DEHALOGENASES**

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### **Background**

Reductive dehalogenases (RDases) are key enzymes in organohalide respiration (OHR) as they catalyze the reduction of halogenated compounds such as tetrachloroethene (PCE), a major environmental pollutant. RDases contain a corrinoid and two iron-sulfur cofactors and require cofactor assembly and maturation prior to transport across the cytoplasmic membrane via the Twin-arginine translocation (Tat) pathway. Recently, a new dedicated Tat molecular chaperone, PceT, has been identified that seems to act in the maturation of the PCE RDase (PceA) of *Dehalobacter restrictus* and members of *Desulfitobacterium hafniense*.

### **Objectives**

This work intends to study the molecular action and specificity of PceT and other members of the RdhT chaperone family on the maturation of RDases.

### **Methods**

Heterologous co-expression of the *pceA* and *pceT* genes will be investigated in *E. coli* in various growth and induction conditions in order to evaluate the effect of PceT on the production, stability and cofactor load of PceA. Additional general molecular chaperones will be also considered here. A detail characterization of the protein-protein interaction between PceT and PceA will be obtained by a combination of *in vitro* biochemical approaches. Site-directed mutagenesis of conserved residues of RdhT proteins should allow identifying key amino acids in the recognition of, i.e. action on, PceA.

### **Conclusions**

Preliminary data already suggested that PceT mainly recognizes the Tat signal peptide of PceA, therefore resembling the paradigmatic Tat specific chaperones (TorD, NapD,...) as they play a major role in quality control of their cognate redox enzyme allowing sufficient time for cofactor assembly and folding.

**FEMS-3017**

**Bacterial cell biology**

**IDENTIFICATION OF A PUTATIVE CHROMOSOMAL REPLICATION ORIGIN FROM *BDELLOVIBRIO BACTERIOVORUS* AND ITS INTERACTION WITH THE INITIATOR PROTEIN DnaA**

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**Background**

*Bdellovibrio bacteriovorus* is a small Gram-negative, obligate predatory bacterium that attacks and invades other Gram-negative bacteria, including pathogens such as *Helicobacter pylori* or *Pseudomonas aeruginosa*. Its life cycle consists of two stages – non-replicative attack phase, wherein predator searches for prey and replicative growth phase, wherein it actively divides in host periplasm. Initiation is the first and strictly regulated step of bacterial chromosome replication, which leads to duplication of the genetic material in bacterial cells prior to their division.

**Objectives**

Our aim was to identify and characterize the key elements of initiation of chromosome replication in *B. bacteriovorus*: origin of chromosomal replication (*oriC*) and initiator protein.

**Methods**

Using *in silico* analysis, we identified the *oriC* region, which is located downstream of the *dnaA* gene; the *B. bacteriovorus oriC* (*BdoriC*) contains eight putative DnaA boxes. Comprehensive *in vitro* studies using EMSA, DMS footprinting and SPR revealed that the DnaA protein specifically binds all eight DnaA boxes. By P1 nuclease assay we localized the DNA unwinding elements (DUE) where DNA replication starts. In addition, we compared the architecture of the DnaA–*oriC* complexes (orisomes) in homologous (*oriC* and DnaA from *B. bacteriovorus*) and heterologous (*BdoriC* and DnaA from prey, *E. coli* or *P. aeruginosa*) systems. Interestingly, we demonstrated that DnaA proteins from preys (relatively distantly related from *B. bacteriovorus*) not only specifically bind *BdoriC*, but also unwind DNA at the DUE.

**Conclusions**

To conclude, we identified the *oriC* of *B. bacteriovorus* and characterize in details its interaction with the replication initiator protein.



**FEMS-0981**

**Bacterial cell biology**

## **LINK BETWEEN PTS<sup>Ntr</sup> OF BRUCELLA ABORTUS AND ITS CENTRAL METABOLISM**

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### **Background**

*Brucella*, an ALPHA2-proteobacteria, is the etiological agent of brucellosis that generates a worldwide zoonosis. It can infect human and will lead, if not treated, to a chronic infection with severe complications.

To proliferate, bacteria need to get carbon sources from their environment and the phosphotransferase system (PTS) contributed to this goal. It displays regulating functions in carbon metabolism and forms a phospho-relay, ending with the entry and concomitant phosphorylation of a sugar.

*Brucella abortus* possesses a paralogous system called Nitrogen PTS (PTS<sup>Ntr</sup>), acting the same way except that no sugar entry occurs. Starting from the phosphoenolpyruvate, the phosphoryl group is successively transferred on histidine residues of Enzyme I (EI<sup>Ntr</sup>) (*ptsP* gene), then to the NPR (*ptsO*) protein and finally to EIIA<sup>Ntr</sup> (*ptsN*) or EIIA<sup>MAN</sup> (*ptsM*).

### **Objectives**

We were interested in the identification of a link between central metabolism and the *B. abortus* PTS<sup>Ntr</sup>.

### **Methods**

We have generated the knockout mutant of those 4 protagonists and studied their bacterial growth behavior on media containing different single carbon source. We found that *B. abortus* was mainly able to grow on sugar entering the pentose phosphate pathway. Interestingly, compared with the wild type strain, the *ptsP* (EI<sup>Ntr</sup>), *ptsO* (NPR) and *ptsN* (EIIA<sup>MAN</sup>) mutants shows a growth defect on those sugars while the *ptsM* (EIIA<sup>Ntr</sup>) mutant grows better. Moreover, point mutation study on NPR protein shows that phosphorylation state of the histidine residue seems to be the main actor controlling growth behavior.

### **Conclusions**

It suggests a role of the *Brucella* PTS<sup>Ntr</sup> in the regulation of central metabolism.

**FEMS-0958**

**Bacterial cell biology**

**ENRICHMENT FOR G1-ARRESTED DAUGHTER CELLS OF BRUCELLA ABORTUS**

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**Background:** In HeLa cells and RAW264.7 macrophages, the facultative intracellular pathogen *B. abortus* blocks its growth and its cell cycle at the G1 stage during the first hours of infection (M. Deghelt *et al.*). G1 bacteria are proposed to have an increased ability to invade these host cells. Therefore, it would be interesting to prepare a bacterial population enriched with G1-arrested daughter cells to investigate *B. abortus* cell cycle in culture and in interaction with the host cells.

**Objectives:** Searching the best method for synchronizing the cell cycle of *B. abortus* at the G1 stage. Then, testing G1 bacteria for their potential invasion of host cells.

**Methods:** Nutrient downshift method was used to produce G1-arrested cells of *Sinorhizobium meliloti* (De Nisco *et al.*). Our results indicate that this is not an effective way to synchronize G1-arrested daughter cells of *B. abortus*. However, culture of *B. abortus* in rich medium until the early stationary phase resulted in a reproducible enrichment in G1 bacteria (about 70%), as assessed by flow cytometry. Another method called “baby machine”, that immobilize a mixed population of bacteria labelled with NHS-biotin (*N*-hydroxysuccinimido-biotin) by streptavidin-coated magnetic beads, was set up to recover only G1 bacteria. We expected that the unipolar growth will be resumed on the beads in rich medium, allowing the enrichment of daughter cells at the G1 stage of the cell cycle.

**Conclusions:** Culture of *B. abortus* in rich medium yielded a partial and transient enrichment of G1 bacteria. It seems that the unipolar nature of growth is no more detectable when bacteria are labelled with NHS-biotin. Other labelled compounds for “baby machine” method are currently tested and analysed to achieve G1 bacteria of *B. abortus*.

**FEMS-0797**

**Bacterial cell biology**

## **CHARACTERIZATION OF LMO1521 AND LMO2591 MUREIN HYDROLASES FROM LISTERIA MONOCYTOGENES**

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### **Background**

*L. monocytogenes* is a gram-positive, foodborne pathogen causing listeriosis with a high mortality rate (1). This species is widespread in nature which is connected with a high number of surface proteins. Very important class of these proteins are murein hydrolases (autolysins), involved in several crucial processes, including: cell growth, turnover of cell wall components, cell separation and division, biofilm formation, protein secretion, autolytic activity of some antibiotics and pathogenicity (2). This work was supported by a grant from the National Center of Science 2013/09/B/NZ6/00710

### **Objectives**

Determination of the activity and substrate specificity of two autolysins from *L. monocytogenes*: Lmo1521 and Lmo2591.

### **Methods**

Lmo1521 and Lmo2591 proteins with C-terminal hexa-His-Tags were expressed in *E. coli* BL21 using expression vector pET-28a (Novagen). These proteins were purified on Ni-NTA Agarose column (Qiagen), and the isolation of the correct proteins was verified by immunoblotting. The effect of overexpression of the proteins on host cells morphology (*E. coli* BL21) was tested using scanning microscopy. To investigate activity we have performed spectrophotometric and zymographic assay (using different purified bacterial cell walls).

### **Conclusions**

Bioinformatic analysis showed the presence of a N-acetylmuramoyl-L-alanine domain in Lmo1521, N - acetylmuramidase domain in Lmo2591 and GW-motif in both. Our results indicate muralytic activity of the studied proteins against different bacterial cell walls. We have also determined the substrate specificity of these two autolysins.

**FEMS-1607**

**Bacterial cell biology**

**THE EFFECT OF RSD, THE ANTI-SIGMA FACTOR OF SIGMA 70, ON BIOFILM FORMATION AND MOTILITY IN ESCHERICHIA COLI**

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**Background**

In bacteria,  $\sigma$  subunit of RNA polymerase (RNAP) directs transcription initiation. The regulation of  $\sigma$  activity is important for fine tuning of gene expression.  $\sigma$  activity is determined by their cellular level, affinity for core RNAP, and interactions with regulatory proteins. In *Escherichia coli*, housekeeping  $\sigma$  factor,  $\sigma^{70}$ , has the highest affinity for core RNAP and is the most abundant  $\sigma$  factor. Rsd, regulator of sigma D, binds specifically to  $\sigma^{70}$  and it has been known as an anti- $\sigma$  factor of  $\sigma^{70}$ .

**Objectives**

Even though Rsd is known as an anti- $\sigma^{70}$  factor, no specific phenotype has been associated with deficiency or overexpression of Rsd to date. In this study, we found new phenotypes of the *rsd* mutant.

**Methods**

Cell-to-cell autoaggregation assay

Biofilm formation assay

Semi-solid agar assay for assessment of motility

**Conclusions**

An *rsd*-deficient mutant cell sank much faster than wild type. In spite of its increased cell aggregation, biofilm formation decreased in the *rsd* mutant. We found a protein of ~ 43 kDa whose expression was significantly higher in the *rsd* mutant compared to wild-type. Peptide mass fingerprinting revealed that this enriched protein is antigen 43 (Ag43) encoded by *agn43*, whose transcription is  $\sigma^{70}$ -dependent. Also, one protein band increased in wild-type and Rsd-overexpressing strains compared to the *rsd* mutant. Mass spectrometry identified this protein band as the flagellin protein FliC. Based on these results, we propose that Rsd decreases the transcriptional level of *agn43* through the regulation of  $\sigma^{70}$  activity and consequently it influences the biofilm formation and motility.



**FEMS-3067**

**Bacterial cell biology**

## **STRUCTURAL CHARACTERIZATION OF THE DNA UPTAKE MACHINERY IN GRAM-POSITIVE BACTERIA**

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### **Background**

Horizontal Gene Transfer (HGT) is an evolutive mechanism by which entire genes are transferred among bacterial cells, thus enabling an immediate adaptation to new environmental conditions. HGT has obvious implications for human health as it is used by pathogenic microorganisms – even among different species– during the spread of virulence factor and antibiotic resistance. Three main routes can be distinguished: *conjugation* (plasmid transfer through the direct interaction of two bacterial cells), *transduction* (bacteriophage-mediated DNA transfer) and *transformation* (uptake of naked DNA from the environment). The term DNA Uptake Pump (DUP) refers to the translocating machinery used by bacteria to incorporate naked DNA from the environment to the cytoplasm during bacterial transformation.

### **Objectives**

The specific aim of this project is to determine the structure of the DNA uptake pump responsible for bacterial transformation in gram-positive bacteria using X-ray crystallography. This machinery is mainly formed by a DNA receptor, a transmembrane channel that mediates DNA translocation across the cytoplasmic membrane and an ATPase which pulls DNA into the cytoplasm at the expense of ATP.

### **Methods**

We adopted a high-throughput approach to achieve our goals. We designed 96 constructs from five different species with several solubility-enhancing fusion partners and affinity tags. The constructs can be divided into three groups: putative soluble constructs, fragments containing trans-membrane helices and full-length integral membrane proteins.

### **Conclusions**

We already have cloned the target genes and have tested them for soluble expression in *E. coli*. Large scale purification and preliminary crystallization trials are in progress for the soluble constructs.

**FEMS-2166**

**Bacterial cell biology**

## **CELL SHAPE CONSERVATION AND REGULATION IN BACTERIA**

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### **Background**

Why bacteria have evolved and maintained their specific shapes is one central question in bacterial cell biology. Rod-shaped bacteria cells are remarkable in keeping their geometry and shape. How this shape is regulated is still mostly uncharted territory.

### **Objectives**

We are setting out to understand how and if rod-shaped bacteria are maintaining their rod shape when subjected to mechanical deformation. We explore how mechanical force changes the bacteria morphology and the consequences for bacteria after the mechanical force is released. The behaviour of proteins, that are involved in maintaining rod-shape, e.g. MreB, Mbl, is analyzed microscopically to understand if mechanical deformation can influence protein localization.

### **Methods**

With the help of soft lithography we are applying microfabrication tools to create microenvironments to manipulate the shape of bacteria. To analyse the shape morphology and protein localization we are using different types of microscopy and automated image analysis tools.

### **Conclusions**

Rod-shaped bacteria are maintaining their rod shape in response to mechanical deformations. Cells return to their original shape with a fast rate in cell wall recovery. It appears that the rod shape is a strong evolutionary feature and tightly controlled.

**FEMS-1152**

**Bacterial cell biology**

**GLYCAN FORAGING SYSTEMS SIGN THE ADAPTATION OF  
CAPNOCYTOPHAGA CANIMORSUS TO THE DOG'S MOUTH.**

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**Background**

*Capnocytophaga canimorsus* (Cc) are Gram-negative bacteria that are part of the normal flora of dogs' mouth and can cause rare but severe infections in humans which have been in contact with a dog.

Cc grow in co-culture with eukariotic cells by harvesting surface glycans via the PUL5 encoded Gpd complex. PUL5 mutant bacteria show a reduced growth and display coccoid forms, which strongly resemble *E. coli* mutant bacteria that are unable to synthesize aminosugars, and are deficient in peptidoglycan synthesis.

We thus speculated that Cc's arrest of growth and cell rounding would be due to starvation of aminosugars.

**Objectives**

Here we address the question why Cc rely on host aminosugars and how they retrieve them from their niche, the dog's mouth.

**Methods**

Cc strain 5 (Cc5) genome analysis revealed that Cc5 could not synthesize N-acetylglucosamine (GlcNAc) because of the lack of two enzymes which convert glucosamine into GlcNAc. Heterologous expression of these enzymes as well as supplementation with GlcNAc completely restored the growth and abolished the formation of coccoid forms.

**Conclusions**

We show that *C. canimorsus* cannot synthesize GlcNAc and thus rely on exogenous GlcNAc which they retrieve by foraging glycans from mucin and N-linked



glycoproteins through two different apparatuses, Muc and Gpd, both related to the *Bacteroides* starch utilization system (Sus).

We propose that the GlcNAc auxotrophy reflects the adaptation of *C. canimorsus* to its host and ecological niche, the dog's mouth.

**FEMS-1465**

**Bacterial cell biology**

## **STREPTOMYCES PEUCETIUS VAR. CAESIUS AND ITS GLUCOKINASES: WHAT THEIR PHYSIOLOGICAL RELEVANCE IS?**

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### **Background**

*Streptomyces peucetius* var. *caesius*, a relevant industrial strain overproduces the antitumor, doxorubicin. As reported for other secondary metabolites, doxorubicin production is also repressed by glucose. In streptomycetes, the glucokinase (ATPGlk), participates in the process of carbon catabolite repression (CCR). However, the mechanism by which this enzyme exerts its regulatory role, has not been elucidated. In this bacterium, we have reported the presence of an additional glucokinase (PPGlk), which uses polyphosphate as its phosphate donor group. Contrary to what is observed for other *Streptomyces* such as *S. peucetius*, *S. coelicolor*, *S. lividans* and *S. thermocarboxydus*, in *S. peucetius* var. *caesius*, PPGlk activity is higher compared to that of ATPGlk. A similar situation has been reported for a chlortetracycline overproducer strain of *Streptomyces aureofaciens*. So far, the implications of the presence of two glucokinases in development and physiology of the genus *Streptomyces* are unknown.

### **Objectives**

To evaluate the role of both glucokinases in the physiology of *S. peucetius* var. *caesius*, their participation in the CCR, morphological development and synthesis of anthracyclines.

### **Methods**

ATPglk::aac(3)/V and PPglk::aadA mutants were obtained by replacing the genes for an apramycin and a streptomycin resistance cassette (*aadA*), respectively. Strains were grown in different carbon sources, and their ATPGlk, PPGlk, b-galactosidase and glycerol kinase activities were determined. Total anthracyclines and morphological differentiation (microscopy) were also evaluated.

### **Conclusions**

The ATPglk::aac(3)/V mutant showed inability to grow in high glucose concentrations and exhibited deregulation in CCR. In regard to the parent strain, the mutant growth and production of anthracyclines, was delayed on solid medium.

**FEMS-1002**  
**Bacterial cell biology**

## **REGULATION OF DIFFERENTIATION AND ANTIBIOTIC PRODUCTION IN STREPTOMYCES GRISEUS**

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### **Background**

The genus *Streptomyces* comprises soil-dwelling, filamentous bacteria having great biotechnological significance since these bacteria produce 75% of the known antibiotics. Autoregulatory molecules play a key role in controlling antibiotic production and morphological differentiation. In *Streptomyces griseus* A-factor (2-isocapryloyl-3R-hydroxymethyl-γ-butyrolactone) proved to be significant as the A-factor negative (AFN) mutant had a non-differentiating phenotype.

### **Objectives**

Previous studies showed that the A-factor biosynthetic *afsA* gene was functional and transcribed in the AFN strain. Our aim is to understand the background of the AFN phenotype.

### **Methods**

In this study the production of AfsA protein, aerial mycelium, spores, extracellular protease and antibiotics was followed on rich and minimal medium. To detect the presence of AfsA by Western blotting we produced the AfsA as a GST-fusion protein in *Escherichia coli*. The purified AfsA was used for immunization of rabbits.

### **Conclusions**

The AFN strain did not form aerial mycelium on rich medium due to the lack of A-factor but spores were formed from the substrate mycelium. Aerial mycelium emergence and sporulation were observed on minimal medium in AFN although the production of spores, extracellular protease and antibiotics were decreased compared to the control strain. The AfsA protein was detected in the AFN strain. These data confirm the hypothesis that the presence of AfsA is not enough for normal A-factor production and A-factor is essential for the aerial mycelium but not for spore formation on rich medium. Moreover our data support the existence of a crosstalk between the A-factor mediated and the starvation induced pathway.



**FEMS-1606**

**Bacterial cell biology**

**DECIPHERING THE METABOLISM OF THE GLYCAN LIPID CARRIER, THE UNDECAPRENYL-PHOSPHATE**

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**Background**

The biogenesis of bacterial cell-wall polymers (e.g., peptidoglycan, lipopolysaccharides, teichoic acids) requires undecaprenyl-phosphate (C<sub>55</sub>-P). The glycan units are linked to this lipid at the inner face of the membrane. Thereafter, the membrane intermediate is translocated in order to transfer the glycan unit to the periplasmic side, where the glycan moiety is transferred to the growing polymer. The process releases undecaprenyl-pyrophosphate (C<sub>55</sub>-PP). C<sub>55</sub>-P originates from the dephosphorylation of C<sub>55</sub>-PP, itself generated by *de novo* synthesis and recycling.

**Objectives**

Identification and biochemical characterisation of bacterial C<sub>55</sub>-P phosphatase.

**Methods**

Genetics: construction of deletion mutants. Biochemistry: membrane protein purification and characterisation (enzymatic activity, membrane topology, mutagenesis, structure).

**Conclusions**

Two families of membrane proteins exhibit C<sub>55</sub>-PP phosphatase activity: BacA and members of the PAP2 super-family. *Escherichia coli* possesses one BacA and three PAP2 proteins (PgpB, YbjG and LpxT), raising the question of the significance of such a multiplicity. LpxT catalyses the transfer of the C<sub>55</sub>-PP phosphate group onto the lipid A moiety of the lipopolysaccharides (LPS), yielding C<sub>55</sub>-P and lipid A-1diP. The role of this LPS modification is under investigation (e.g., antibiotic resistance, ion homeostasis, innate immunity). This raises a tantalising hypothesis that the other C<sub>55</sub>-PP phosphatases could also exhibit such a phospho-transferase activity with different acceptor molecules. Our research project is aimed at the complete functional and structural characterisation of these multiple membrane phosphatases.



**FEMS-2359**

**Bacterial cell biology**

**1MDa CELL DIVISION PROTEIN COMPLEX IN ESCHERICHIA COLI AS REVEALED BY NATIVE PAGE**

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**Background**

Bacterial cell division is carried out at mid-cell position by a group of cell division proteins referred to as the divisome. For the Gram-negative model bacterium *Escherichia coli*; ten of these proteins (FtsZ, -A, -K, -B, -L, -N, -Q, -I, -W and ZipA) are considered as the core players of division. Thus far, interaction networks between individual divisome proteins have been investigated extensively with use of various biochemical and genetic techniques and many interactions between cell division proteins have been found. Still, no oligomeric interactions have been demonstrated that show that the divisome is present as a large multi-protein machinery.

**Objectives**

To detect whether or not a complete 'divisome' assembly is present in the cell.

**Methods**

Clear Native gel Electrophoresis on mildly solubilized *E. coli* cells, followed by 2D Electrophoresis and Western Blotting.

**Conclusions**

We describe the finding of a large 1 MDa cell division protein complex in *E. coli*, that includes at least 8 essential division proteins; FtsZ, ZipA, FtsK, FtsQ, FtsB, FtsL, and FtsN. The complex is present in cultures of rapidly dividing cells, but not when cells are not dividing. Also, we found that a slight overexpression of *ftsQ D237N* (encoding interaction-impaired FtsQ), prevents formation of this 1 MDa complex. Combined, our findings indicate that a large protein complex containing cell division proteins indeed exists.

**FEMS-1007**

**Bacterial cell biology**

**GAMMA-GLUTAMYLTRANSPEPTIDASE EXPRESSION IN CAMPYLOBACTER JEJUNI IS NOT ONLY REGULATED BY THE RACRS TWO-COMPONENT SYSTEM**

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**Background**

*Campylobacter jejuni* is an asaccharolytic human pathogenic Gram-negative bacterium that utilizes amino acids as carbon and energy source and colonizes the gastrointestinal tracts of warm-blooded animals. Some strains of *C. jejuni* possesses the enzyme gamma-glutamyltranspeptidase (GGT), which enables growth on glutathione and glutamine by generation of glutamate in the periplasm and contributes to persistent colonization of the avian gut.

**Objectives**

We have shown that the RacRS two-component system is involved in cytoplasmic glutamate anabolism by regulating the *gltBD* genes, here we investigated whether the RacRS system also regulates the periplasmic glutamate anabolism by regulating the *ggt* gene.

**Methods**

Our results show that RacRS regulates the expression of *ggt* under low oxygen conditions. Under these conditions, higher transcription levels of the *ggt* gene and enhanced GGT activity are observed in the wt compared to the *racR* mutant strain. By using different *ggt* truncated promoter elements in EMSA and luciferase reporter assays we show that RacR binds directly on a specific location of the *ggt* promoter. Furthermore we show that under high oxygen conditions *ggt* expression peaks around end-log phase and GGT activity is highest in stationary phase and is not dependent on RacR. GGT expression and activity is repressed by addition of glutamine to the medium or glutamine catabolic products.

**Conclusions**

In conclusion we show that the *C. jejuni* GGT activity is dependent on multiple factors and one of them is the RacRS two-component system.





**FEMS-1692**

**Bacterial cell biology**

## **EFFECT OF HUMAN ANTIMICROBIAL PEPTIDES AGAINST LEGIONELLA PNEUMOPHILA**

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### **Background**

Antimicrobial peptides (AMPs) are natural antibiotics widespread throughout the animal kingdom, from bacteria to mammals. They are important components of both innate and adaptive immunity, providing protection against a broad-spectrum of pathogens, such as viruses, bacteria, fungi and parasites.

### **Objectives**

Investigate the effect of two human AMPs (HBD-3 and LL-37) on *Legionella pneumophila*

### **Methods**

In this study, we investigated the action of synthetic LL-37 and HBD-3 on both extracellular and intracellular lifestyle of *L. pneumophila*.

### **Conclusions**

We showed that both peptides exhibit a phase dependent bactericidal effect on extracellular *L. pneumophila*. We also observed that LL-37 and HBD3 inhibit intracellular replication of *L. pneumophila* in macrophages and pneumocytes. We showed by colony counting assays that the adherence and internalization of *L. pneumophila* was not affected in presence of LL-37, but was stimulated with HBD-3, suggesting that these two peptides restrain the bacterial replication by different mechanisms of action.

Human AMPs LL-37 and HBD-3 seem to be involved in innate immunity against *L. pneumophila* by acting on the extracellular bacteria and on its intracellular replication.



**FEMS-0754**

**Bacterial cell biology**

**SINGLE-MOLECULE IMAGING REVEALS HETEROGENEOUS MOBILITY OF  
MEMBRANE PROTEINS IN LIVE ESCHERICHIA COLI**

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**Background**

Membrane proteins perform vital cellular functions like respiration, signaling and nutrient uptake. For proper functioning, conformational dynamics, complex formation and ability to diffuse in the membrane are vital parameters. Despite a lot of work on model membranes, little is known about lateral mobility of proteins in bacteria.

**Objectives**

To investigate how molecular crowding, protein size and membrane fluidity affect the mobility of membrane proteins in living *E. coli* bacteria

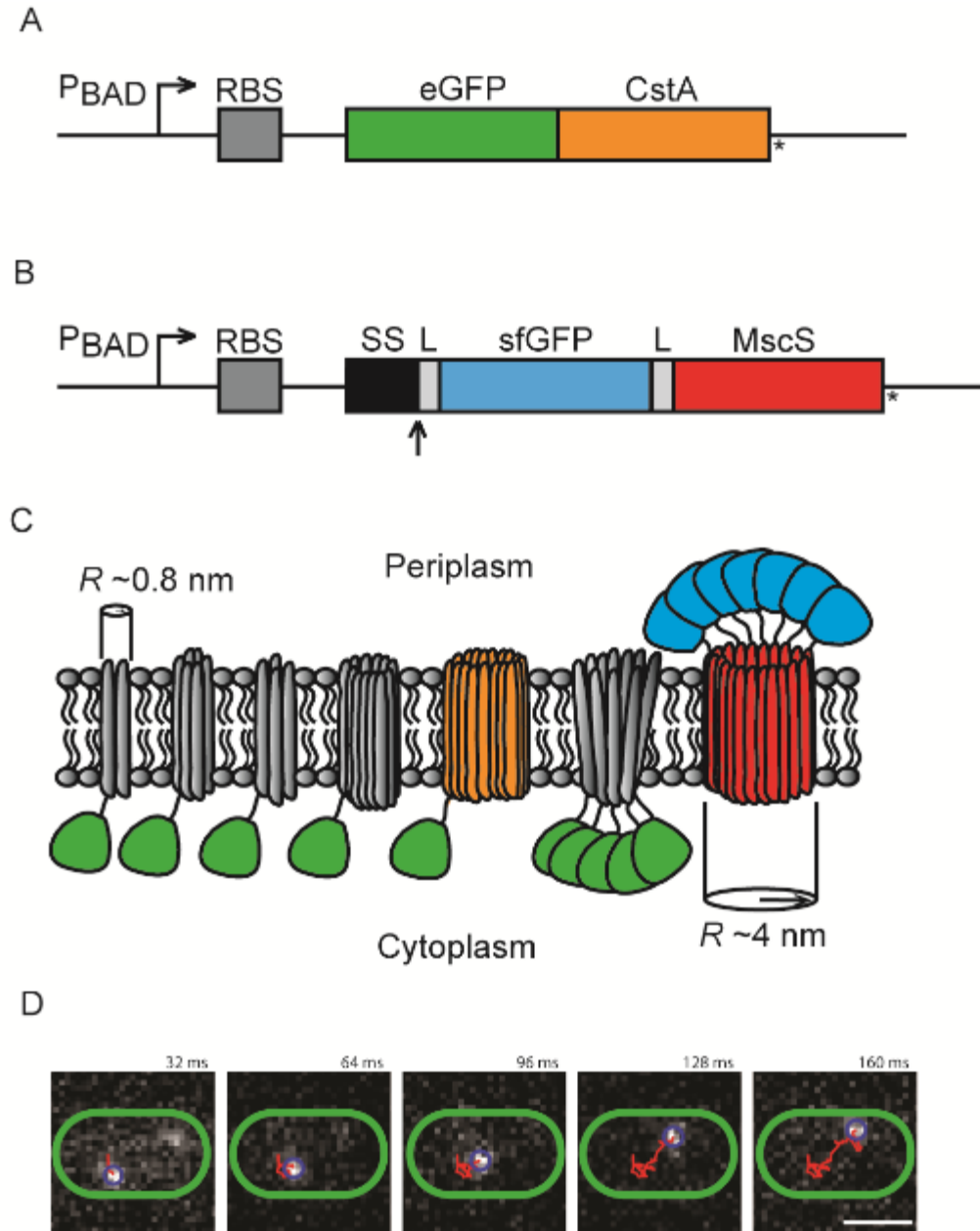


Fig. 1. (A) Schematic diagram of expression construct for the production of cytoplasmic fluorescent protein (FP). fluorescent protein (eGFP) is represented in green, orange indicates peptide transport protein (CstA). Arrow points to the site of leader peptidase processing. RBS, ribosome-binding site. The asterisk indicates a stop codon. (B) Schematic diagram of expression construct for the production of periplasmic exported fluorescent protein (FP). Signal peptide (SS) used to export is represented in black, gray represents the linker (L), blue indicates fluorescent protein (sfGFP), red indicates mechanosensitive channel protein of small conductance (MscS). Arrow points to the site of leader peptidase processing. RBS, ribosome-binding site. The asterisk indicates a stop codon. (C) Schematic representation of a biological membrane, consisting of seven *E. coli* trans-membrane proteins embedded in phospholipids. (D) Time-laps images of single ssDsbA-sfGFP-MscS transmembrane protein tracked in the inner membrane of an *E. coli* cell (scale bar: 1  $\mu$ m)

## Methods

Here we use single-molecule wide-field epi-fluorescence microscopy to track the

lateral mobility of seven integral membrane proteins of different size fused to green fluorescent protein in living *E. coli*. We apply a novel method, IPODD to extract accurate diffusion coefficients from the 2-D projected diffusion trajectories along the 3-D curved bacterial membrane

### **Conclusions**

The diffusion coefficients we find are significantly lower than those reported in *in vitro* studies of isolated membrane proteins in GUVs. Our results indicate that molecular crowding in the plasma membrane of *E. coli* substantially slows down trans-membrane protein diffusion. Surprisingly, all seven proteins studied diffuse heterogeneously: they all show a faster and a slower moving component. Our interpretation of the heterogeneity is that it reflects heterogeneity in membrane composition. To further support this interpretation, we stained *E. coli* inner membrane with a lipid-mimicking dye Dil-C12 and tracked its lateral mobility. Strikingly, Dil-C12 diffuses heterogeneously yet again reflecting the dynamic heterogeneous nature of the plasma membrane. Therefore, from these observations, we propose that the plasma membrane of *E. coli* might contain micro-domains with different fluidity that could play key roles in specific localization of proteins.

**FEMS-0876**

**Bacterial spores**

**WHEN MICROBIAL SURVIVAL STRATEGIES NEVER STOP TO WONDER:  
SPORULATION OUTSIDE FIRMICUTES**

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**Background**

Spore or spore-like structures are only found in four bacterial phyla: Actinobacteria, Cyanobacteria, Proteobacteria and Firmicutes. These structures provide resistance to adverse conditions. The ability to form spores is not, however, a widely spread characteristic and it is restricted to only some orders within those phyla. For example amongst Proteobacteria, solely  $\delta$ -proteobacteria can produce spore-like fruiting bodies, or so we knew. A novel  $\gamma$ -proteobacterium, *Serratia ureilytica* str. Lr5/4, was found to produce spores that not only resemble structurally to those produced by endospore-forming Firmicutes, but also provide heat-resistance.

**Objectives**

The aim of this study is to describe this novel strain and its Firmicute-like spores and to reveal the molecular pathway of this sporulation procedure in comparison to those that are already known.

**Methods**

Physiological, biochemical, carbon source assimilation and antibiotic resistance tests were performed. Morphology of vegetative cells and spores was described by phase contrast microscopy, SEM, and TEM. Moreover, spores of Lr5/4 were revived after heat-shock tests and shown to contain dipicolinic acid (DPA). These two characteristics were so far unique to the heat-resistant endospores found in Firmicutes. Sequencing and annotation of its full genome has been performed in order to reveal the relationship of spore formation in Lr5/4 to other known sporulation pathways.

**Conclusions**

It has been previously proposed that the properties of spore formation in non-sporulating species were due to molecular gene transfer. However, the present study rejects this scenario and demonstrates a novel mechanism for the formation of the described spores of *S. ureilytica*.





**FEMS-1489**

**Bacterial spores**

**BACILLUS SUBTILIS BIOFILMS INCREASE GUT COLONIZATION AND LIFESPAN IN THE ANIMAL MODEL CAENORHABDITIS ELEGANS**

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**Background**

Biofilm proficiency has a great impact in bacteria-host interactions. *Caenorhabditis elegans* and the spore-forming bacteria *Bacillus subtilis* are both common soil inhabitants which are used as models of lifespan studies. However, the role of *in vivo* biofilm development during gut colonization and its effects on animal lifespan are poorly documented.

**Objectives**

Here, we investigated the ability of *B. subtilis* to form a biofilm in the intestine of *C. elegans* and its effects on gut colonization and lifespan of the nematode.

**Methods**

The results showed that wild-type NCIB3610 strain was able to colonize and persist in the gut of *C. elegans* more efficiently than the laboratory strain JH642. The ability to make a biofilm was essential for the observed behavior because FICT-labelled NCIB3610-derived biofilm-mutant cells, but not FICT-wild -type cells, lost the capacity to persist and colonize the nematode gut. *In situ fluorescence microscopy* and beta-galactosidase expression, driven from the promoter of surfactin harbored by *B. subtilis* engineered cells and used to feed the worm confirm the higher ability of NCIB3610, instead of JH642 cells, to colonize the gut of *C. elegans*. The ability to make a persistent biofilm correlated well with a positive lifespan effect of the bacterium on the nematode.

**Conclusions**

*B. subtilis* spores are able to germinate, growth, make biofilma and persist in the intestine of *C. elegans*. In addition, we provide evidences showing that the intestinal life cycle of *B. subtilis* making a biofilm is beneficial and required for the increase for the lifespan of *C. elegans*.

**FEMS-1492**

**Bacterial spores**

**CHARACTERIZATION OF SPORE-FORMING BACILLUS, ISOLATED FROM THE ANDES MOUNTAINS, EXHIBITING HIGH LEVELS OF RESISTANCE TO ARSENIC AND UV RADIATION**

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**Background**

The North-Western part of Argentina is particularly rich in wetlands located in the Puna at an altitude between 3,600 and 4,600 m above sea level. Here, incidence of high levels of UV radiation and contamination with high contents of toxic metals, particularly arsenic (As) is common. We hypothesize that one strategy to contribute to the remediation of these area could be the use of *Bacillus* spp.

**Objectives**

We collected soil and water samples from different parts of the Puna and isolated native spore-forming Bacilli. The novel isolates were examined at different growth temperatures and osmolarities on their ability to grow and make biofilms in the presence of toxic concentrations of As and high UV radiation.

**Methods**

The Bacilli communities were characterized by pyrosequencing 16S rRNA gene amplicons derived from specific PCR oligonucleotides. The Andean *Bacillus* isolates displayed a greater capacity to make persistant biofilms, colonize surfaces (swarming motility) and tolerance to high temperatures, salinity and UV-B / UV-C radiations at levels that were lethal to common laboratory strains.

**Conclusions**

The present results allow the potential biotechnological application of the isolated *Bacillus* strains with bioremediation potential of pristine environments contaminated with metals.

**FEMS-1989**

**Bacterial spores**

## **NOVEL GENES INFLUENCING THE GERMINATION RATE OF SPORES OF BACILLUS SUBTILIS FOOD ISOLATES**

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### **Background**

*Bacillus subtilis* responds to starvation with formation of endospores. Spores can easily contaminate food materials and their resistance impedes their removal from food products. In response to various triggers, spores reinitiate vegetative growth via germination. Vegetative cells can subsequently cause food spoilage. Accurate prediction of germination behavior is difficult as differences in germination responses are observed even among various strains of one species. Thus, data obtained for the model strain, *B. subtilis* 168, cannot be directly applied to food-spoiling strains.

### **Objectives**

The purpose of this study was to elucidate germination behavior of spores of *B. subtilis* strains that cause food spoilage and to couple the observed germination phenotypes with the strains' genomic contents.

### **Methods**

Genomes of thirteen *B. subtilis* industrial isolates were sequenced. Germination of their spores was induced under various conditions and analyzed via absorbance measurements and phase-contrast microscopy. The spore germination responses of different strains were linked with their genomic content using gene-trait matching software.

### **Conclusions**

Strains were divided into two phenotypic groups based on the ability of their spores to germinate, in particular their germination rates. Slower germination correlated with the presence of specific genes in industrial isolates, which are absent in the laboratory strain *B. subtilis* 168. Insertion of these genes into *B. subtilis* 168 decreased the rate of germination of its spores. Thus, new genes influencing *B. subtilis* germination responses were identified. The function of these genes is currently being elucidated. These genes can be used in the future as biomarkers indicating slow germination.

**FEMS-3099**

**Bacterial spores**

## **UNDERSTANDING BACTERIAL SPORE RESISTANCE TOWARDS PLASMA STERILIZATION**

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### **Background**

Background: Spores of *Bacillus subtilis* have been used extensively as biological indicators for industrial purposes such as sterilization or decontamination. For several years a number of activities in the field of sterilization of heat sensitive materials by means of non-thermal plasmas have been known. Plasma sterilization methods are characterized by the use of gas or gas mixtures that are partially excited by an applied electric field. Plasma discharges contain a high degree of UV/VUV-radiation, as well as charged particles and free radicals, which exert detrimental effects on microorganisms by damaging genetic material, outer cell layers and proteins.

### **Objectives**

Objectives: We are interested in understanding the protective attributes and molecular mechanisms involved in the *B. subtilis* spore resistance to plasma sterilization.

### **Methods**

Methods: In a systematic manner different *B. subtilis* spores varying in their protection (e.g., coat, crust, DPA, SASP formation) and DNA repair capabilities were studied on their impact towards plasma sterilization.

### **Conclusions**

Conclusions: We will present our recent findings on the protective attributes and molecular mechanisms involved in the spore resistance to plasma sterilization. The multi-layered proteinaceous spore coat, being the first barrier to environmental influences, was shown to be a major factor contributing to spore resistance towards plasma treatment. During spore revitalization, DNA damage accumulated during the dormant spore stage is the subject of a variety of different repair systems. Deficiencies in repair genes of non-homologous end-joining, spore photoproduct

lyase, or nucleotide excision repair led to a significant decrease in spore resistance to low pressure plasma sterilization.

**FEMS-2471**

**Bacterial spores**

**SPORULATION TEMPERATURE HAS LIMITED EFFECT ON THE SPORE PROTEOME OF BACILLUS WEIHENSTEPHANENSIS.**

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**Background**

*Bacillus weihenstephanensis* is a spore forming food pathogen known for its ability to sporulate, germinate, grow and produce toxins at lower temperatures than its close relatives. As such, it presents an increased risk to food safety where standard procedures rely on cold-inactivation of microbes. Properties of the infective form Bacilli, the spore, are known to vary with the conditions under which the spores were produced. For instance, lower sporulation temperature has been indicated to result in a lower wet heat resistance. This resistance is in part attributed to the spore coat layers, proteinaceous layers surrounding the core which contains the bacterial DNA.

**Objectives**

The aim of this study was to determine the proteomic basis for the heat resistant phenotype. As no specific singular protein can be indicated to confer heat resistance, this supposedly acquired trait can only be appreciated by analysis of the spore proteome as a whole.

**Methods**

Analysis of the proteome of spores of *B. weihenstephanensis* strain WSBC10204 produced at either 12°C or 30°C by mass spectrometry provided insight into variations in the protein content of the spore coat at different sporulation temperatures.

**Conclusions**

Interestingly, only minor differences were observed, suggesting wet heat resistance does not depend on the identity of the proteins making up the coat layers. As wet heat resistance was indeed lower for the spores produced at lower temperatures, low temperature might instead affect the rate of spore maturation instead, a process where spore coat proteins are cross-linked after they have been deposited onto the spore.

**FEMS-1185**

**Bacterial spores**

**CARBON FIXATION AND ALLOCATION IN A FILAMENTOUS  
CYANOBACTERIUM DURING AKINETE DIFFERENTIATION USING NANOSIMS  
AND MICROSCOPY**

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**Background**

Akinetes are resting cells of species members of the Nostocales and Stigonematales orders of cyanobacteria. These are spore-like, non-motile cells that differentiate from vegetative cells and serve a perennating role. During their differentiation, akinetes varies their metabolic activities and cellular pools.

**Objectives**

Here we follow carbon assimilation in the cyanobacterium *Aphanizomeno ovalisporum* and accumulation of cyanophycin during akinetes differentiation and maturation.

**Methods**

Photosynthetic assimilation of  $\text{H}^{13}\text{CO}_3^-$  was studied by NanoSIMS technology and variations in abundance and distribution of cyanophycin globules were followed by transmission electron microscopy and histochemistry techniques.

**Conclusions**

NanoSIMS results clearly indicate that during their differentiation, akinetes maintain metabolic activity and assimilate inorganic carbon via photosynthesis. Towards advanced stages of differentiation and maturation, the metabolic activity of akinetes reduced and many of the free akinetes lost their photosynthetic capacity as indicated by changes in  $^{13}\text{C}/^{12}\text{C}$  ratio. Nevertheless, a small group of free (mature akinetes) still maintains their photosynthetic capacity. Structural changes during akinetes differentiation observed by transmission electron microscopy and histochemistry techniques, included the accumulation of cyanophycin bodies in akinetes. The rate of cyanophycin accumulation and carbon allocation into this protein storage is currently being investigated in a complementary NanoSIMS analysis.

**FEMS-3062**

**Bacterial spores**

**THE ESSENTIALS OF LIFE; PROTEOMICS OF DORMANT AND AWAKENING SPORES OF BACILLUS SUBTILIS**

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**Background**

In response to nutrient limitation Gram positive organisms like *Bacillus subtilis* form dormant spores. These cellular entities are survival capsules, resistant to chemical and environmental assaults. They pose challenges to the food and medical sectors. Upon contact with germinants spores return to vegetative life through a process called 'germination and outgrowth'. The vegetative cells may cause food spoilage and food borne diseases.

**Objectives**

To perform a detailed quantitative time-resolved proteomics study of (dormant)spores and their secretome during germination and outgrowth

**Methods**

Using a quantitative proteomics approach a study of the time resolved break down of spore proteins during early stages of germination was initiated. Spores were germinated in minimal medium supplemented with a germinant mixture (L-Asparagine, D-glucose D-fructose and Potassium Chloride). The spore layers and the secretome were analyzed separately by Ion Trap LC-MS/MS analysis

**Conclusions**

During germination the action of proteases, lytic enzymes and peptidases in the spore results in proteolysis, cortex hydrolysis and break down of spore dormancy. Partially digested proteins as well as intact proteins are released from the spore in to the medium. Study of these proteins by our method allowed us to understand molecular details of the germination process. Current analyses are aimed at correlating the germinating spore proteome to its transcriptome under control as well as thermal and organic acid stress conditions (Keijser et al. 2007 and Ter Beek et al., our unpublished observations). We aim at deconvoluting heterogeneous germination and outgrowth profiles using our live imaging technology (Pandey et al., 2013).





## **FEMS-1450**

### **Bacterial spores**

#### **STRAIN DIVERSITY ALONG THE LIFE CYCLE OF BACILLUS CEREUS**

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#### **Background**

Undomesticated spore formers isolated from the environment or from processing facilities commonly show higher diversity, resistance and more heterogeneous behaviour compared to widely used reference strains domesticated in laboratories and that may have lost relevant traits. Analysis of such undomesticated isolates therefore provides valuable insights in sporulation capacity, spore resistance, germination and outgrowth efficiency that determine survival and fitness in different environmental niches including food and food processing environments.

#### **Objectives**

In our project we aim to obtain insight in the diversity among pathogenic *Bacillus cereus* strains with focus on spore properties. To this end, *B. cereus* reference strains ATCC14579 and ATCC10987 were analysed and compared to behaviour of strains isolated from food and food production facilities.

#### **Methods**

Growth, sporulation and germination capacity of 20 *B. cereus* strains isolated from different food products and two reference strains was characterized under different conditions. Spore stress resistance and germination characteristics were assessed upon exposure to disinfectants, heat and mild processing technologies. Behaviour of untreated and treated spores was analysed both at population level as well as at the level of individual spores by using Flow cytometry (FCM) in combination with sorting (FACS).

#### **Conclusions**

The *B. cereus* strains displayed a highly diverse response with respect to growth, sporulation, spore resistance and germination capacity. Additionally, spore population behaviour showed substantial differences with some strains displaying a

homogenous and other a heterogeneous performance. We have demonstrated the relevance of including environmental isolates in the studying behaviour of pathogenic spore former *B. cereus* along the different steps of life cycle.

## FEMS-2565

### Bacterial spores

#### INSIGHT IN HEAT INACTIVATION AND GERMINATION BEHAVIOR OF HEAT RESISTANT SPORES OF BACILLUS LICHENIFORMIS, B. AMYLOLIQUEFACIENS AND B. THERMOAMYLOVORANS

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### Background

*Bacillus licheniformis*, *B. amyloliquefaciens* and *B. thermoamylovorans* can cause significant non-sterility issues in high heat treated foods due to survival of heat resistant spores that can subsequently germinate followed by outgrowth during the shelf life.

### Objectives

To gain improved control of spores of these species in food manufacturing, a genetic basis for spore heat resistance and germination efficiency was investigated by combining spore phenotypes and genome information of individual strains.

### Methods

The heat inactivation kinetics of spores of different isolates were determined. In addition, the genomes of over 20 strains within these species were sequenced to investigate a genetic basis for different spore heat resistances.

For *B. licheniformis* (10 strains) and *B. amyloliquefaciens* (9 strains) significant differences in heat resistance of spores were found for different isolates. Strains producing heat resistant spores contained a subset of genes belonging to a cluster that confers high heat resistance in *B. subtilis*.

For spores of *B. thermoamylovorans* (4 strains) spore heat resistance, nutrient germination, non-nutrient germination, and detailed spore counts were determined. Non-nutrient germination using calcium-dipicolinic acid induced good germination of spores of all strains. Poor germination on regular rich cultivation media resulted in gross underestimation of the viable spores truly present.

## **Conclusions**

The above findings shed light on different phenomena that can contribute to spore survival. Such information is important to devise appropriate strategies to enumerate spores in food ingredients and to inactivate spores, leading to improved control of heat resistant spores in foods.

**FEMS-2151**

**Bacterial spores**

**SSGB COORDINATES SEPTUM SITE LOCALIZATION WITH CHROMOSOME ORGANIZATION DURING STREPTOMYCES SPORULATION**

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**Background**

Streptomyces are multicellular bacteria with a complex life cycle. During the reproductive phase aerial hyphae transition into chains of hundred or more spores via a highly orchestrated cell division process. Coordination of sporulation is regulated by the SALP proteins (SsgA-like proteins). SsgA forms an array of foci along the hypha and provides an anchor for SsgB to multimerize on the cell membrane, after which SsgB recruits FtsZ and stimulates FtsZ polymer formation.

**Objectives**

SsgB is a highly conserved protein that forms trimers. The C-terminal  $\alpha 3$  helix is important for trimerization and interacts with  $\alpha 1$  and  $\alpha 2$  of the neighboring SsgB monomers. To elucidate how SsgB interacts with SsgA and FtsZ, we have created an SsgB mutant library.

**Methods**

These ssgB variants were introduced in the ssgB mutant strain, after which strains were automatically scored for restored sporulation via a scanner based imaging approach. Over 500 variant SsgB a single amino acid change and these were further analysed.

**Conclusions**

All sporulating samples were examined with transmission electron microscopy (TEM), which revealed effects varying from changes in spore wall thickness, size distribution, DNA segregation to affecting spore shape. In contrast wild type spores are similar sized, have condensed DNA in the center of the spores and have a thick spore wall.

We have shown that single amino acid changes in SsgB can facilitate many division related phenotypes. and ultimately dictate septum orientation resulting in longitudinal division. The latest results in elucidating the function of individual amino acids will be presented.

**FEMS-0486**

**Biofilms**

**EVALUATION OF TWO SCREENING METHODS FOR DETECTION OF BIOFILM FORMATION AND ITS EFFECT ON ANTIBIOTIC SUSCEPTIBILITY AMONG STAPHYLOCOCCI SPECIES**

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**Background**

Biofilm formation is a hallmark characteristic of staphylococcal infections as it allows attachment to solid surfaces, exchange of genes that can result in more virulent strains, production of high concentration of toxins, evasion of immune defenses and antimicrobial resistance.

**Objectives**

This study was conducted to evaluate two different screening methods for detection of Biofilm formation and its effect on antibiotic susceptibility among the clinical isolates of different Staphylococci species.

**Methods**

**Seventy five isolates of staphylococci were tested by modified tissue culture plate method (MTCP), congo red agar (CRA) method and tube method (TM) for their ability to form biofilm. Antibiotic susceptibility testing was done for thirty isolates, 15 biofilm-producers and 15 biofilm nonproducers, using broth microdilution assay.**

**Conclusions**

The rate of detection of biofilm formation among the staphylococcal isolates by the MTCP method, the CRA method and the TM were 48%, 38.7% and 25.3% respectively. Comparing to the results of MTCP method as a gold standard, the sensitivities of the CRA method and tube method were 80.6% and 52.8% respectively. Both showed 100% specificity. Regarding the antibiotic susceptibility of the biofilm producers and non-producers, statistically significant differences were detected for oxacillin, ceftriaxone and augmentin susceptibility ( $P < 0.02$ ). All the

isolates were sensitive to vancomycin. The MTCP takes 3 days and many steps. The TM is observer dependent. Congo red agar method can be used as a screening method for detection of biofilm formation among staphylococcal isolates as it is cheap, rapid with no subjective errors and requires less expertise.



**FEMS-1037**

**Biofilms**

**INFRARED SPECTROSCOPIC ANALYSIS OF XYLELLA FASTIDIOSA BIOFILMS**

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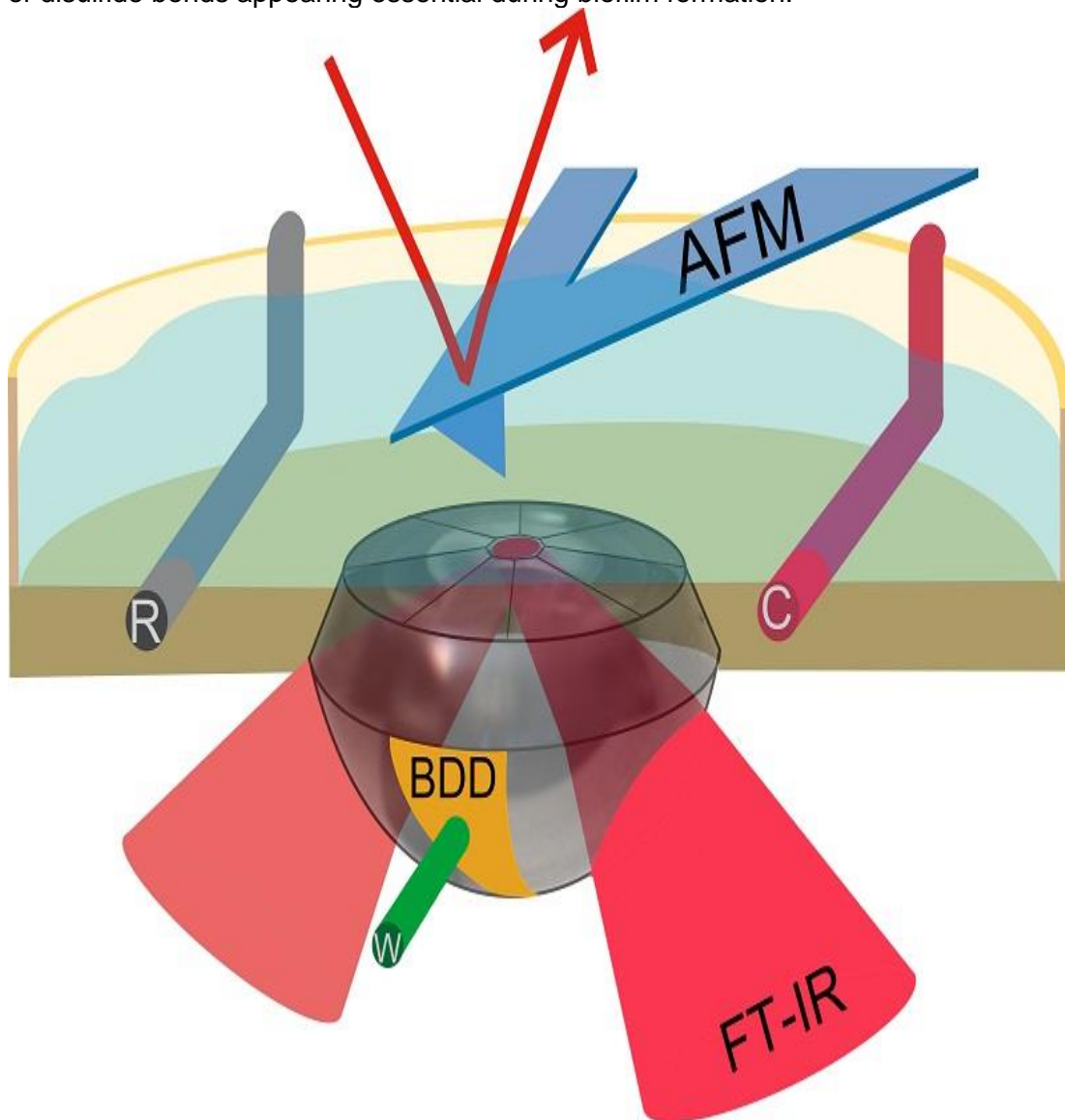
**Background**

*Xylella fastidiosa* (*Xf*) affects plant xylem vessels, causing diseases such as Pierce's disease. As abundant plants of economic relevance may be affected by *Xf*, it is essential understanding the biofilm formation behavior of *Xf*, whose genome has been sequenced partly by our collaborators for developing appropriate countermeasures.<sup>[1]</sup>

**Objectives**

The appearance of *Xf* infected olive trees in Italy is of substantial concern due to the first appearance of this species within Europe. Considering the role of biofilms within many human diseases, the investigation of the adsorption behavior of bacteria along with the involved biomolecules is evident.<sup>[2][3]</sup> Particularly interesting is the formation

of disulfide bonds appearing essential during biofilm formation.<sup>[4]</sup>



### Methods

This study focuses on the analysis of biomolecules involved in *Xf* biofilm formation via vibrational spectroscopy. In a unique instrumental combination, infrared attenuated total reflection (IR-ATR) spectroscopy and atomic force microscopy (AFM) were applied to characterize the growth media and biofilms associated with *Xf*.

### Conclusions

Experimental and analytical procedures will be discussed providing first insight on the involved molecular processes during biofilm formation and maturation.

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**FEMS-0580**

**Biofilms**

## **SUPPLEMENTARY MICROBIOLOGICAL ANALYSIS OF FILLING MATERIALS REVEALS NEW INSIGHT INTO THE ECOLOGY OF PERSISTENT ENDODONTIC INFECTIONS**

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### **Background**

Persistent root canal infections correlate with endodontic treatment failures. Microorganisms have been found in 35%-100% of previously treated root canals. The analysis of the microbiota of endodontic infections is prerequisite for improvement of treatment methods of infected root canals.

### **Objectives**

1. The microbiological analysis of infected root-filled teeth with periradicular lesions using culture-dependent and -independent approaches
2. The analysis of the microorganisms adhered to the retrieved endodontic filling materials (EFM) and the comparison with the results gained from the root canal samples alone.

### **Methods**

Twenty patients were enrolled in this study and samples from the corresponding twenty root-filled teeth were taken according to standard protocols. In addition to these samples, the EFM were also analyzed microbiologically using culture and culture-independent methods and transmission electron microscopy. The culture-independent technique was conducted by cloning and sequencing of 16S rRNA amplicons.

### **Conclusions**

**Results:** Bacteria were revealed in 15 root canal samples and in 18 EFM samples at a concentration ranging from  $10^3$  to  $10^7$  cfu/ml. 56 different species were found in association with the root canal filling materials. In contrary, only 47 species could be identified in the root canal samples. 20 species were detected solely in the EFM samples including *Aggregatibacter actinomycetemcomitans* and *Eubacterium nodatum*. The most abundant species belonged to the phyla Firmicutes, Actinobacteria and Proteobacteria.

**Conclusions:** The analysis of microorganisms associated with root-canal filling materials in addition to the root canal samples taken after revision reveals a better insight into endodontic infections and should not be neglected.

**FEMS-2580**

## **Biofilms**

### **ANALYSIS OF INTERACTIONS BETWEEN NEISSERIA STRAINS IN BIOFILMS**

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#### **Background**

The human nasopharynx is colonized by different species of the genus *Neisseria*, including commensals, such as *N. lactamica*, but also an important pathogen, i.e. *N. meningitidis*. In situ, these bacteria are organized in micro-colonies, structures that resemble biofilms and that may represent a defense mechanism for the bacteria to survive in a hostile environment. In vivo, these species must interact and compete for the same niche.

#### **Objectives**

Here, we designed novel constructs to generate green and red fluorescent *Neisseria* strains to facilitate the discrimination between different microorganisms by microscopy in several assays.

#### **Methods**

These constructs were stably integrated in a highly conserved region on the chromosome of both species, as evidenced PCR assays, and allowed for constitutive expression of the fluorescent proteins. We next optimized biofilm formation assays and studied the organization of biofilms of independent and mixed *Neisseria* strains.

#### **Conclusions**

Our results showed that indeed biofilms of monocultures of *N. meningitidis* and *N. lactamica* are organized as micro-colonies with intervenient spaces. However, the organization of these spaces and the distribution of the biomass varied between strains of the same species. Mixed biofilm assays revealed that both species can differently interact, altering the organization of the biofilms. This work is the first report of the interaction between both species and allows for speculation about the process of human colonization.

**FEMS-1919**

**Biofilms**

## **CHANGES IN SEWER BIOFILM MICROBIAL COMMUNITIES RELATED TO DOWNSTREAM NITRATE DOSAGE**

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### **Background**

Nitrate ( $\text{NO}_3^-$ ) is an effective and widely used chemical in sewer systems to reduce biogenic sulfide ( $\text{H}_2\text{S}$ ) and methane ( $\text{CH}_4$ ) produced in anaerobic rising main pipes. However, the main limitation of this approach is that anoxic conditions must be continuously kept through the whole pipe, otherwise  $\text{H}_2\text{S}$ -producing biofilms prevail.

### **Objectives**

The aim of this study is to determine the effects of the Downstream Nitrate Dosage strategy (DND) on anaerobic sewer biofilms with regards to sulfide oxidation and, for the first time, on methane production.

### **Methods**

Effectiveness of the strategy was assessed on  $\text{H}_2\text{S}$  and  $\text{CH}_4$  abatement on the effluent of specially designed laboratory-scale systems that mimics a real sewer biofilms. A combination of process (normal functioning monitoring and batch tests) and molecular (by 454-pyrosequencing) methods were used to investigate the impacts and microbial activities related to the nitrate addition.

### **Conclusions**

Results showed a complete abatement of  $\text{H}_2\text{S}$  generated, due to the growth of a Sulfide-Oxidizing Nitrate-Reducing population. Methane was reduced to 50% while nitrate was added, due to the  $\text{CH}_4$  oxidation in the anoxic conditions established at the end of the pipe. An increase of microorganisms of the genera *Simplicispira*, *Comamonas*, *Azonexus* and *Thauera* was detected during nitrate addition. Regarding anoxic methane oxidation, only one Operational Taxonomic Unit (OTU) was identified, which is likely related with this metabolism. Both sulfidogenic and methanogenic activities resumed upon cessation of  $\text{NO}_3^-$  dosage. Obtained results are relevant for the optimal management of nitrate dosage strategies in sewer systems.





**FEMS-1933**

**Biofilms**

## **CHANGES OF MICROBIAL COMMUNITIES DURING DIFFERENT STAGES OF BIOFILM DEVELOPMENT IN SEWER SYSTEMS**

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### **Background**

Transport of wastewater along sewer pipes facilitates the formation of microbial biofilms that grow attached to their inner surfaces. Anoxic conditions in sewers favour the production of both sulfide (H<sub>2</sub>S) and methane (CH<sub>4</sub>) as end products of anaerobic microbial metabolisms, i.e. sulfate-reduction and methanogenesis, respectively. The build-up of H<sub>2</sub>S and CH<sub>4</sub> in sewerage causes different detrimental effects such as odour, corrosion and toxicity.

### **Objectives**

The aim of this study was to investigate the initial stages of development of microbial biofilms in sewer systems with a special focus on the interactions between sulfate-reducing bacteria (SRB) and methanogenic archaea (MA).

### **Methods**

The work was carried out using a laboratory sewer pilot plant fed with wastewater that mimic the functioning of a real anaerobic pressured sewer. Biological activities and phylogenetic community composition were investigated during sewer colonisation using a combination of molecular techniques (DGGE, qPCR and 16S rRNA tag sequencing) and process data (H<sub>2</sub>S and CH<sub>4</sub> production).

### **Conclusions**

Results showed that the SRB community was established and active after two weeks of biofilm development, causing substantial sulfide accumulation in the pilot plant. In turn, growth of MA and methane production were low during initial stages of biofilm colonisation but readily increased over time after the replacement of initial MA species derived from human faeces (*Methanobrevibacter* spp.) by other MA representatives more adapted to sewer conditions (*Methanosaeta* spp.). Altogether, our results pointed to different dynamics and activity of key community members from sewer biofilms, greatly affecting the production kinetics of H<sub>2</sub>S and CH<sub>4</sub> along time.



**FEMS-2943**

**Biofilms**

**LIPIDOMICS OF THE BIOFILM FORMED BY VIBRIO CHOLERAЕ N16961 UNDER INORGANIC PHOSPHATE LIMITATION IN THE PRESENCE OF BILE SALT**

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**Background**

To better understand the molecular events that occur during *Vibrio cholerae* biofilm formation *in vivo*, we did a previous proteomic analysis of planktonic and biofilm cells, after 14, 24 and 48h of growth under conditions that mimic the intrainestinal environment, namely, pH 7.4 at 37°C, Pi limitation and the presence of the bile salt, sodium deoxycholate (DOC). The groups of differential proteins identified indicated that free cells were at stationary phase, whereas, those in biofilms were at exponential culture phase, after 48h growth. Moreover, in planktonic cells at all times, alkaline phosphatase was produced, suggesting Pho regulon activity, which was not observed in the biofilms cells.

**Objectives**

To perform a lipidomic analysis to explore differences in lipid profiles between planktonic and biofilm cells of *V. cholerae*.

**Methods**

Cells were grown overnight in MGLP (MOPS, salts, glucose, pH 7.4 with KH<sub>2</sub>PO<sub>4</sub> at 0.65μM-low phosphate) without (free cells) or with 0.2% DOC (biofilm) for 14-48hs, at 37°C, without agitation. The lipids were extracted according to Bligh and Dyer 1959 and analyzed by Q-TOF LC/MS.

**Conclusions**

In the 14h planktonic cells, the great majority of phospholipids was replaced by ornithine containing lipids (OL). Between 24 and 48h, OLs were found in these cells, but in smaller amounts. In biofilm cells, phosphatidylethanolamine (PE) was the major lipid at all times, whereas OLs were detected in small amounts. These data suggest, for the first time, the involvement of PhoB regulated genes in the expression regulation of OLs and PE in *V. cholerae*.

**FEMS-2362**

**Biofilms**

**ADHESION OF ENTEROCOCCUS FAECIUM ON PLASTIC AND STAINLESS STEEL AND ITS ANTI-ADHESIVE POTENTIAL TOWARDS PATHOGENS**

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**Background**

Bacteria have the ability to attach to surfaces commonly found in the food processing environment, such as polystyrene and stainless steel and to become more resistant to environmental stresses and sanitizers. So the search for new fight strategies is needed

**Objectives**

In this study, a strain of *Enterococcus* (*En.*) *faecium*, originally isolated from a milking machine inner-surface, was studied for its biofilm formation potential on plastic and stainless steel supports and its antibacterial and anti-adhesive potential against four pathogens (*Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853 and *Listeria innocua* CLIP 74915).

**Methods**

The adhesion and the anti-adhesive potential of this strain were studied (at 25 and 37°C) using polystyrene microtiter plates and stainless steel 304 L coupons after their surface characterization by contact angle method.

**Conclusions**

The results revealed that the strain was a strong biofilm producer on polystyrene and stainless steel either at 25 and 37°C with the best adherence level observed at 25°C. *En. faecium* cells and culture supernatant showed a significant ( $p<0.05$ ) inhibition potential of the pathogens most probably due to bacteriocins synthesis.

**FEMS-0712**

**Biofilms**

**BIOFILMS X-TREME: TOLERANCE OF THE THERMOACIDOPHILIC ARCHAEON SULFOLOBUS ACIDOCALDARIUS TO 1-BUTANOL EXPOSURE**

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**Background**

Archaeal biofilms are interesting for biotechnological applications like whole cell biocatalysis, because they promise a higher tolerance towards toxic compounds, which is a characteristic property known for biofilms. In this study the effect of the organic solvent 1-butanol on biofilms of the thermoacidophilic, aerobic crenarchaeon *Sulfolobus acidocaldarius* with growth at 78 °C and pH 3 was investigated. *S. acidocaldarius* is adapted to extreme environments, genetically tractable and able to form biofilms.

**Objectives**

The aim of this study was to characterize the tolerance of *S. acidocaldarius* biofilms to 1-butanol exposure. The effect to 1-butanol on biofilm formation as well as on established biofilms was investigated.

**Methods**

Submerged biofilms were grown (4 d, 78 °C) in 96-well microtiter plates, Petri dishes or  $\mu$ -dishes on either polystyrene or glass surfaces. 1-butanol was present during biofilm formation or added to already established biofilms. Analysis of biofilms was performed by microscopic methods (confocal laser scanning microscopy, epifluorescence microscopy, atomic force microscopy, environmental scanning electron microscopy), and microbiological and biochemical methods (determination of total cell counts, viable counts and aerobic respiration activity). Extracellular polymeric substances were isolated and quantified by colorimetric assays.

**Conclusions**

*S. acidocaldarius* tolerates 1-butanol concentrations up to 1.5 % [v/v] as validated by viability assays. 1-butanol exposure led to stress responses like aggregation of cells, a change of carbohydrate distribution and composition as well as carbohydrate and

protein amounts in the biofilm matrix. Butanol-exposed biofilm cells revealed alterations of shape and loss of cell appendages.

**FEMS-0937**

**Biofilms**

**EFFECT OF THAI-PLANT EXTRACTS AND COMBINATION WITH ANTIBIOTICS  
ON PSEUDOMONAS AERUGINOSA BIOFILM FORMATION**

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**Background**

*Pseudomonas aeruginosa* is the most common Gram-negative bacteria causing fatal nosocomial infections in hospitalized patients especially with the immunocompromised subjects. Bacteria can resist a wide variety of currently available antibiotics. One of resistant mechanisms is by forming the biofilm in infected host preventing drugs to access to the respective targets of the bacterial cell. Novel strategy to overcome this antibiotic resistant mechanism is to use the combination of antibiotics with inhibitors of biofilm formation.

**Objectives**

The objective in this study is to investigate the effect of mulberry-leaf, fruit and goji berry on biofilm formation from *P. aeruginosa*.

**Methods**

The result was shown by specific biofilm formation (SBF) index and the ethanolic extract (256 mg/ml) and the ether extract (32 mg/ml) of mulberry-leaf presented strong activity (SBF index > 1.1) to reduce biofilm in 44.0 % and 44.8 %, respectively. The plant extract with anti-biofilm activity was tested further in combination with gentamicin (0.125 to 1024 µg/dl) by Checkerboard method indicated that there was no synergistic efficacy. The results of MBEC and CLSM revealed that the ethanolic extract (32 mg/ml) was effective on *P. aeruginosa* biofilm.

**Conclusions**

The extract of mulberry-leaf could be an alternative thai herbs for biofilm formation inhibiting the most common drug resistant bacteria.

**FEMS-0904**

**Biofilms**

## **BIOFILMS FORMATION IN LABORATORY-SCALE ANAMMOX UPFLOW BIOREACTOR DURING PROLONGED CULTIVATION**

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### **Background**

In natural and man-made ecosystems, anammox bacteria demonstrate a strong tendency for attached growth and biofilms formation.

### **Objectives**

We studied biofilms structure and formation by microbial community with dominant group of anammox bacteria, enriched in anaerobic vertical upflow bioreactor with nitrogen load 5 g N/l per day and upflow vertical concentration gradient of nitrogen substrates and pH.

### **Methods**

Biofilms structure and microbial community composition were studied via microscopy (light, electron, confocal and atomic force) and molecular genetics methods (sequencing and FISH). Biofilm formation *de novo* was studied on microscopic slides submerged in the upper part of the bioreactor.

### **Conclusions**

Several groups of microorganisms are involved in biofilm formation. Anammox bacteria belong to three different species. Their satellites are: trichal forms (presumably *Chloroflexi*), coccoid and rod-shaped cells (likely different species of nitrifier and denitrifier communities involved in nitrogen removal and trace amounts of oxygen consumption). *Chloroflexi* community includes 3 phylotypes of *Anaerolineae* and *Dehalococcoidia* strains responsible for biofilm spatial structure formation and trace amounts of organics elimination.

Primary biofilms are discovered on day 10-12. Subsequent growth of biofilms appears due to increasing the number of cells involved in biofilms and extracellular polymeric matrix accumulation. Mature biofilms (80-90 days) include cells of the same morphotypes as mature biofilms inside the reactor: clustered cocci with typical



anammox ultrastructure, interstitial rods and remarkably long trichal cells (more than 100  $\mu\text{m}$  long).

Surface microrelief images of such biofilms were obtained via atomic force microscopy.

**FEMS-1574**

**Biofilms**

**ASSESSMENT OF BIOFILM DESTABILIZATION AND REMOVAL BY LAUNDRY AGENTS**

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**Background**

The formation of biofilms is a well known phenomenon in a variety of technical and natural environments such as rivers, water systems or washing machines. They can harbor a potential risk to human health serving as habitat for pathogens. Furthermore, biofilm growth within washing machines leads to biocorrosion and unpleasant odours. Therefore, the removal or prevention of biofilm growth is of great interest.

The relevance of biofilm EPS in terms of bacterial adhesion to surfaces has been indicated in several studies. Therefore, EPS can be one working point for laundry agents for the destabilization and detachment of biofilms.

**Objectives**

The objective of this investigation was to test the efficacy of different laundry agents on biofilms.

**Methods**

Biofilms grown in rinse water were treated with cleaning bases in a flushing chamber. Several concentration and fluxes of cleaning bases were tested. The attack of the cleaning agents on EPS components was analyzed by confocal laser microscopy after simultaneous staining of bacteria, proteins and polysaccharides.

**Conclusions**

Our findings showed that cleaning agents did not contribute an attack on specific polymer-groups of the EPS. The selection of the detergent as well as the applied shear forces were of rather great impact. Laundry agents containing oxidative bleach were much more effective than agents containing low or non bleach. CLSM images revealed the compact biofilm structure changed into a small- sized structured biofilm. Worst results were obtained by using liquid detergents. Furthermore, shear forces were of greater impact than the flushing duration and supported the effects of cleaning agents.



**FEMS-2477**

**Biofilms**

## **RESPONSE OF SESSILE CELLS OF CANDIDA SPECIES TO OXIDATIVE STRESS**

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### **Background**

The biofilms of *Candida* species are associated with high indices of hospital morbidity and mortality [1,2]. Sessile cells are highly resistant to antifungals and to the host mechanisms of defense [1,3-5]. Though the ability of some *Candida* species to form biofilms on various medical implants has been evaluated as well as their response to oxidative stress [4,6], it is however important to investigate how the reactive oxygen species impacts on sessile cells of different *Candida* species.

### **Objectives**

To evaluate the response of oxidative stress on biofilm formation by *C. albicans*, *C. glabrata*, *C. krusei* and *C. parapsilosis*.

### **Methods**

Sessile and control cells of *Candida* species were exposed to increasing concentrations of H<sub>2</sub>O<sub>2</sub>. Biofilms were observed by scanning electron microscopy and quantified by the XTT assay.

### **Conclusions**

To our knowledge, this is the first report describing the effect of oxidative stress on biofilm formation by four *Candida* species *in vitro*. Our results suggest that candidiasis *in vivo* is a multifactorial and complex process where the human immune system and the adaptability of the pathogen should be considered altogether to provide an effective treatment of the patient.

**FEMS-3155**

**Biofilms**

## **TRANSCRIPTIONAL REGULATION OF THE FUNCTIONAL AMYLOIDS IN PSEUDOMONAS**

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### **Background**

The *fap* operon is present in the majority of *Pseudomonas* species and encodes for the *Fap* functional amyloid system, which is associated with cell aggregation and enhanced biofilm formation. Six genes make up the operon.

### **Objectives**

However, it is so far unknown how the *fap* operon is regulated.

### **Methods**

In this study, a biotinylated dsDNA probe was constructed in order to isolate potential DNA binding molecules, which specifically associate with the promoter region of the *fap* operon of the amyloid expressing *Pseudomonas* sp. UK4 and potential transcription factors were identified using LC-MS/MS taking advantage of a closed genome sequence. Two global regulators (MvaU and MvaT) were found to interact specifically, with the *fap* promoter. Specific binding of the alternative sigma factor (RpoN) was also observed. The latter observation is interesting as a gene encoding an RpoN activator protein is located next to the *fap* operon. Therefore, we created overexpression and deletion mutants of *Pseudomonas* sp. UK4 with the RpoN activator protein and the two general transcription regulators MvaU and MvaT.

### **Conclusions**

A hydrolysis probe based qPCR assay was developed in order to determine the effect of all these gene regulatory players on the *fap* operon. Consequently, the effect of elevated and decreased levels of c-di-GMP was also studied and experiments will show how these regulators affect transcription of the *fap* operon. This study contributes with an increased understanding of the transcriptional machinery that

control Fap-mediated biofilm formation. In the future, this regulatory system could be an important target in antibiofilm strategies.

**FEMS-3050**

**Biofilms**

## **EFFECT OF DENTAL BIOMATERIALS ON STREPTOCOCCUS MITIS/HUMAN GINGIVAL FIBROBLASTS CO-CULTURE**

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### **Background**

The biocompatibility of oral biomaterials depends not only on the biomaterial properties, but also on the host and oral indigenous microbiota response.

### **Objectives**

A co-culture model of *Streptococcus mitis*/Human Gingival Fibroblast (HGF) was set-up to evaluate the biocompatibility of: 2-hydroxyethylmethacrylate (HEMA), triethyleneglycoldimethacrylate (TEGDMA) and new synthesized bioactive lactose-substituted chitosan-silver-nanoparticles (Chitlac-nAg) in colloidal solution and as thermosets coating.

### **Methods**

*S. mitis* was co-cultured with HGFs with the above mentioned materials in presence of saliva in terms of bacterial adhesion and aggregation, HGF cell toxicity, expression of apoptosis-associated proteins and intracellular signaling.

### **Conclusions**

HEMA treatment showed an increased aggregation and adhesion of *S. mitis* on HGFs, mediated by the PKCa/integrin b1 signalling system. HEMA treatment decreased viable eukaryotic cell that was balanced by the presence of bacteria and saliva.

Concerning TEGDMA, *S. mitis* growth was not decreased and large bacterial aggregates were present on HGFs. Moreover, *S. mitis* and saliva reduced the HGFs oxidative stress and apoptosis induced by TEGDMA treatment.

Chitlac-nAg colloidal solution, in the HGF/*S. mitis* co-culture, induced less numerous viable bacteria adhering to the HGFs, in respect to the control. For coated thermosets, our results showed that the Chitlac-nAg coated thermosets cytotoxicity exerted on HGFs was absent in the co-culture model, where HGFs adhere and migrate on biomaterial.

Taken together, our results shed new light on the interaction of dental biomaterials/Streptococci/HGFs occurring in oral environment, underling the

importance of the co-culture model, resulting closer to the *in vivo* situation, to obtain a more realistic outcome.



**FEMS-2652**

**Biofilms**

**IMPACT OF BIOFILM GROWTH PHASE, DIVERSITY AND EPS QUALITY ON THE REMOVAL EFFICIENCIES OF BIODISPERSANTS USED FOR REDUCING BIOFILM ADHESION IN COOLING TOWERS**

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**Background**

Biocides are commonly used to control unwanted biofilm development in cooling tower. The more environmentally friendly biodispersants can also be used, but little information is available on the efficiency of their application, likely affected by the biofilm structure, composition and growth phase.

**Objectives**

The objective of this work was to study the structure and diversity of biofilms developed from makeup water used in a full scale industrial cooling tower. The efficacy of the biodispersant was further evaluated on biofilms at different growth phase.

**Methods**

Biofilms were grown on glass slides, in a batch system, using as source community the cooling tower makeup water under four different nutrient conditions. The taxonomic bacteria affiliation was investigated by Fluorescence *In Situ* Hybridization (FISH) and diversity index was calculated. EPS were detected *in situ* by epifluorescence microscopy after staining with fluorochromes. A biodispersant commonly utilized in full scale cooling towers was then used and removal efficiencies at different stage of development were evaluated.

**Conclusions**

FISH allowed to identify about 90% of total bacteria, mainly belonging to *Proteobacteria*. Starting from the source community, biofilms showed an initial increase in biodiversity likely due to the functional niche diversification. Then, diversity decreased, mainly due to the adhesion of eukaryotes and to the concurrent reduction in bacteria taxon richness. The removal efficiencies of biodispersant decreased over time likely due to the higher biofilm stability at the late growth stage, where an increase of  $\alpha$  and  $\beta$  glucans, mainly found surrounding eucaryotic and

alphaproteobacterial cells, was observed.

**FEMS-1850**

**Biofilms**

**ΔPPIB ENHANCED MOTILITY AND BIOFILM FORMATION ABILITY IS POSSIBLY MEDIATED THROUGH CHANGES IN THE PPIB INTERACTIONS WITH SOME OF ITS PREY PROTEINS**

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**Background**

*E. coli* ppiB is a peptidyl-prolyl *cis/trans* isomerase with chaperone activity. Δ*ppiB* strain shows enhanced swimming and swarming motility as well as biofilm formation ability.

**Objectives**

We hypothesize that the effects of ppiB on such cellular behaviors are likely accomplished through its protein interactions. Using a candidate approach we aim to identify multicopy suppressors of the Δ*ppiB* phenotypes and to characterize protein interactions with putative ppiB prey proteins.

**Methods**

Based on data provided by IntAct database we considered available ppiB prey proteins as putative interacting partners. To address whether these proteins could be direct effectors inhibiting motility or biofilm formation we over-expressed each of them in the Δ*ppiB* cells and checked for restoration of the corresponding phenotypes.

Further, we examined possible protein interactions with ppiB under native or denaturing prey conditions. To explore the potential involvement of the PPIase active site on the binding of ppiB to these proteins, we determined the inhibitory effect of each of them on the PPIase activity of ppiB.

**Conclusions**

Both the hyper-motility and enhanced biofilm formation ability of Δ*ppiB* strain was reversed by over-expression of certain prey proteins albeit different in each condition. With very few exceptions, we were not able to detect stable protein complexes, probably an indication for weaker transient interactions. However, under denaturing prey conditions, more interactions were revealed suggesting that ppiB is able to recognize denatured preys. A decrease in the ppiB PPIase activity, in the presence of many of them, further supports many of the putative associations.

## EFFECT OF SHEAR STRESS ON PSEUDOMONAS AERUGINOSA ISOLATED FROM THE CYSTIC FIBROSIS LUNG

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### Background

Chronic *Pseudomonas aeruginosa* lung infections are the main cause of morbidity in cystic fibrosis (CF) patients. Previously, we have sequenced the genome of the transmissible Belgian epidemic strain CF\_PA39. This CF-adapted strain has been present in the UZ Brussel hospital environment for at least ten years. Furthermore, earlier experiments showed that culturing *P. aeruginosa* PAO1 in a low fluid shear environment, obtained by means of the Rotating Wall Vessel (RWV) technology, leads to the formation of a biofilm phenotype comparable to that observed in the CF lung.

### Objectives

To study the effect of fluid shear on biofilm formation by an adapted *P. aeruginosa* CF strain in artificial sputum medium (ASM) at the genotypic, transcriptomic, and phenotypic level.

### Methods

In this study, an RWV experiment was performed that closely resembled the *in vivo* situation by inoculating this adapted CF isolate in ASM in the RWV either with or without two glass beads, simulating high and low fluid shear conditions, respectively. Scanning electron microscopy (SEM) was utilized to determine biofilm formation in both conditions, while RNAseq and qPCR was used to study gene expression in both conditions.

### Conclusions

Increasing fluid shear in the RWV model disrupted biofilm formation of an adapted *P. aeruginosa* CF isolate.

In accordance with the biofilm disruption, several genes involved in denitrification, tryptophane synthesis, choline metabolism, and alginate biosynthesis were down-regulated in the high fluid shear condition, resembling the planktonic stage of growth. Furthermore, we identified small RNAs that are differentially expressed.

**FEMS-1526**

**Biofilms**

## **ZINC OXIDE NANOROD PHOTOCATALYTIC COATINGS FOR BIOFILM PREVENTION**

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### **Background**

It has been shown that zinc oxide (ZnO) nanostructures can photocatalytically inhibit growth of fresh water bacterial and fungal strains under solar irradiation.

### **Objectives**

The objective of this study was to investigate the prevention of formation of marine biofilms by ZnO nanorod coatings in the laboratory and out-door experiments.

### **Methods**

ZnO nanorod coatings were fabricated on microscope glass substrates by a simple hydrothermal technique using equimolar solutions of 10 mM zinc nitrate and hexamethyltetramine. In laboratory conditions, upon 5 h of white light irradiation (light intensity= 100 Klx from a tungsten-halogen lamp), nanorod coatings significantly reduced the growth of marine bacterium *Acinetobacter* sp. and inhibited biofilm formation as compared to coatings kept in the dark (not exposed to light). In the continuous 7 days out-door experiment conducted in an aquaria (volume = 70 L) with sea water collected from Oman sea (Muscat, 23°34'55" N, 58°36'27" E), the nanorod coatings significantly reduced density of bacteria in comparison to the control (no coatings). In the absence of sunlight, test and control slides were equally colonized by bacteria. *De novo* sequencing of bacterial biofilms on MiSeq system® demonstrated that different communities were formed in the presence and absence of light on nanorod coatings.

### **Conclusions**

Our study suggests that ZnO nanorod coatings effectively prevent biofilm formation and can be used as a novel green antifouling technology.

**FEMS-2711**

**Biofilms**

**Bacterial Alpha-Amylase Enzyme with Inhibition Effect on Biofilm Formation in Clinical Isolates of *Acinetobacter baumannii***

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**Background**

*Acinetobacter baumannii* is a pathogen with a high quality to form biofilm which can be isolated from different environmental sources. In addition, pathogenicity and biofilm formation associated with infectious agent.

**Objectives**

In this study, we investigated that degradation of biofilm formation by *Acinetobacter baumannii* is determined with amylase enzyme from *Bacillus amyloliquefaciens*.

**Methods**

*Bacillus amyloliquefaciens* is amylase enzyme producer which was obtained from Hacettepe University Department of Biology/Biotechnology. The biofilm forming ability in clinical isolates of *Acinetobacter baumannii* was determined by both microscopic and spectrophotometric analyses with using 24-well polystyrene plates.

**Conclusions**

According to results, amylase which obtained from *Bacillus amyloliquefaciens* was observed to inhibit biofilm formation in *Acinetobacter baumannii*. In continuous, amylase enzyme prevent to increase *Acinetobacter baumannii* cell population. As well as, in most of pathogen bacteria like *Acinetobacter baumannii*, amylase enzyme is considered to degradation of biofilm formation. In a result, amylase enzyme can be used as a drug supplement.

## FEMS-2033

### Biofilms

#### EFFECT OF SUBINHIBITORY CONCENTRATION OF SILVER NANOPARTICLES ON ADHESION OF GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA ONTO POLYSTYRENE SURFACES

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#### Background

In biofilms, bacterial cells adhere irreversibly to a solid surface by extracellular polymeric substances. This way of life provides several advantages over the planktonic mode, as responses to environmental stress.

#### Objectives

The objective was determined the effect of subinhibitory concentration of silver nanoparticles (Ag-NPS) and silver nitrate (AgNO<sub>3</sub>) on adhesion of gram-positive and gram-negative bacteria.

#### Methods

Ag-NPs were synthesized by reduction of AgNO<sub>3</sub> with sodium citrate. Bacterial strains used were gram-positive *Staphylococcus aureus* ATCC 6538, *Bacillus cereus*, isolated from milk cooling tank and *Enterococcus faecalis* ATCC 51299 and gram-negative *Escherichia coli* ATCC 11229, *Salmonella enterica* serovar Typhimurium ATCC 13076 and *Pseudomonas aeruginosa* ATCC 15442. The minimum inhibitory concentration was determined by macrodilution technique according CLSI, 2003. Müller-Hinton broth supplemented with subinhibitory concentration of Ag-NPs or AgNO<sub>3</sub> were transferred to 96-wells polystyrene microtiter plates, and inoculated with 5,0 x 10<sup>5</sup> cfu/ml of cell suspension. After 48 h at 37°C, the optical density was determined at 600 nm and supernatant was discarded. The surface-attached cells were stained with crystal violet for 30 min and washed three times with water. The absorbance at 590 nm was determined by addition of ethanol.

#### Conclusions

Adhesion was estimated by calculating the ratio between the absorbance of violet crystal and the optical density of cells. Subinhibitory concentrations of Ag-NPs and AgNO<sub>3</sub> increased the adhesion of the gram-positive bacteria, probably, as response to stress. The adhesion of *E. coli* and *S. Typhimurium* was not affected by presence of Ag-NPs or AgNO<sub>3</sub>. However, *P. aeruginosa* adhesion decreased in the presence of Ag-NPs.





**FEMS-2440**

**Biofilms**

### **CANDIDA GLABRATA BIOFILMS RESPONSE TO AMPHOTERICIN B**

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#### **Background**

*Candida* species are responsible for recurrent human infections, mostly in immunocompromised patients, due to their high vulnerability. *Candida glabrata* has been showing to have a major role in these infections being the second most prevalent species involved in human fungemia. Amphotericin B (AmB), a common antifungal drug, is a hospital-environment exclusive polyene, normally being efficient when used to fight candidiasis.

#### **Objectives**

The main goal of this work was to infer about the influence of AmB in *Candida glabrata* biofilms formation and its effect on matrix composition and *ERG* genes expression.

#### **Methods**

*Candida glabrata* biofilms were formed in the presence of AmB and analyzed by dry weight. Moreover, *ERG* genes expression was evaluated by qRT-PCR and matrix was analyzed in terms of composition in carbohydrates, proteins, beta-glucans and a new finding: ergosterol.

#### **Conclusions**

In addition to an inefficient reduction of the *C. glabrata* biofilms, this work showed that *ERG* genes seem to be less involved than the matrix composition in *C. glabrata* biofilms response to AmB. Specifically, *C. glabrata* biofilms matrices respond with an increase of carbohydrates, particularly beta-1,3 glucans, and with a decrease of total proteins. The ergosterol values did not expressively changed in the presence of AmB.

The present work support the theory of multifaceted mechanisms developed by *C. glabrata* biofilms as response to the presence of AmB.

**FEMS-0861**

**Biofilms**

## **FLOW CELL ANALYSIS OF THE PROCESS OF BACTERIAL CELL ADHESION MEDIATED BY THE ADHESIVE NANOFIBER PROTEIN ATAA UNDER SHEAR STRESS**

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### **Background**

Elucidation of bacterial adhesion is important for preventing detrimental biofilm formation and for applying it to microbial immobilization. An environmental strain, *Acinetobacter* sp. Tol 5 shows nonspecific, remarkably high adhesiveness to solid surfaces through AtaA (*Acinetobacter* trimeric autotransporter adhesin) nanofibers.

### **Objectives**

We aim to examine the effects of flow rate on the adhesion and autoagglutination of resting Tol 5 cells to reveal adhesion property mediated by AtaA.

### **Methods**

Bacterial adhesion was analyzed through using a flow cell system and the shear stress of the flow was controlled by flow rates and the viscosity of cell suspension supplied with PEG-400. The biomass of attached cells was quantitatively measured by staining with crystal violet. The thickness of the cell clump adhered on the surface was measured by confocal laser scanning microscopy.

### **Conclusions**

Higher shear stress in the flow cell decreased bacterial adhesion and the thickness of cell clump formed on the flow cell surface. By contrast, the bacteria adhesion to well-plates was increased by mild agitation compared with that in a static condition due to enhanced autoagglutination of the cells. These results suggest that there is the optimum flow rate for cell adhesion in the balance between enhancement of autoagglutination due to the increase in the collision probability of bacterial cells and detachment of cells and/or inhibition of cell-cell or cell-surface interaction by shear stress.



**FEMS-2338**

**Biofilms**

**HRPB DEAD-BOX RNA HELICASE IS IMPORTANT FOR MOTILITY, BIOFILM FORMATION AND CANCER DEVELOPMENT IN XANTHOMONAS CITRI SUBSP. CITRI**

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**Background**

DEAD-box RNA helicases are proteins highly conserved, present in all eukaryotic cells and in many bacteria. DEAD-box RNA helicases are involved in various processes of RNA metabolism and play important roles, e.g., translation initiation, ribosome biogenesis and RNA decay. Several bacterial DEAD-box proteins have been studied extensively due to their importance in *E. coli*, *Bacillus subtilis* and *Staphylococcus*. Interestingly, in *Staphylococcus aureus*, the DEAD-box protein CshA was involved in biofilm formation via modulation of *agr* mRNA stability. However, the function of DEAD-box RNA helicases remains unexplored in relation to adhesion, biofilm formation or pathogenicity in *Xanthomonas citri*.

**Objectives**

To characterize the function of DEAD-box RNA helicase (XAC0293/HrpB) in relation to motility, biofilm formation and citrus canker development in *Xanthomonas citri* subsp. *citri*.

**Methods**

We conducted assays for analysis of sliding motility, biofilm formation on biotic surface and pathogenicity of the wild type and the *hrpB* mutant of *X. citri* subsp. *citri*. Expression of different genes between the wild type and mutant strains was determined by quantitative RT-PCR.

**Conclusions**

Deletion of the *hrpB* gene reduced the expression of type IV pili genes, which are required for motility, biofilm development and adherence. Our data show that HrpB regulates the mobility and adherence of the *X. citri* in host. HrpB hereby is the first DEAD-box RNA helicase in *Xanthomonas citri* that is implicated in the regulation of

pili genes and that it is involved in motility, biofilm formation and citrus canker development.

**FEMS-1330**

**Biofilms**

## **COMPARATIVE GENOMICS OF IRON TRANSPORTING SYSTEMS AND FUNCTIONAL ANALYSIS IN BACILLUS CEREUS STRAINS FOR GROWTH AND BIOFILM FORMATION**

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### **Background**

Human pathogens such as *Bacillus cereus* are in constant competition for iron with other microbes and their host. In most environments, including food and mammal hosts, iron is bound to complex compounds and is not readily available. However, pathogenic bacteria developed mechanisms to scavenge iron from a range of sources.

### **Objectives**

The objective of this study was to investigate the use of different complex and non-complex iron sources by *Bacillus cereus* reference strains ATCC14579 and ATCC 10987, and 20 undomesticated food isolates, for which growth phenotypes were linked to genotypes.

### **Methods**

Genome sequences were analysed for the presence of putative iron transport systems. Growth and biofilm formation were monitored in LB with and without iron scavenger Bibyridine and different iron sources. Transcriptome studies in *B. cereus* ATCC10987 revealed upregulation of many putative iron transport systems upon depletion of iron.

### **Conclusions**

All 22 *B. cereus* strains could effectively use Fe citrate and FeCl<sub>3</sub> for growth, and formation of air-liquid interface biofilms was promoted. Hemoglobin and Hemin were used by all, except one strain lacking functional petrobactin and IIsA systems. Interestingly, Hb and Hemin triggered a submerged type biofilm formation by several strains. Ferritin and transferrin could be used for growth only by three and six strains respectively, but biofilm formation was inhibited. Lactoferrin did not restore the growth, however it supported submerged biofilm formation by several strains. Knowledge of iron transport systems and their functioning can serve as an indicator for bacterial fitness in different environments including usage of host-derived iron sources.



**FEMS-0869**

**Biofilms**

**MICROSCOPIC ANALYSIS OF THE PROCESS OF BACTERIAL CELL ADHESION  
AND AUTOAGGLUTINATION MEDIATED BY THE ADHESIVE NANOFIBER  
PROTEIN ATAA**

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**Background**

Elucidation of bacterial adhesion is important for preventing detrimental biofilm formation and for applying it to microbial immobilization. An environmental strain, *Acinetobacter* sp. Tol 5 shows nonspecific, remarkably high adhesiveness to solid surfaces through AtaA (*Acinetobacter* trimeric autotransporter adhesin) nanofibers.

**Objectives**

We aim to reveal the mechanism of cell adhesion mediated by AtaA and describe the cell adhesion process in a microscopic level.

**Methods**

The time profile of cell adhesion was analyzed through confocal laser scanning microscopy (CLSM) and flow cell analysis. The mechanism of autoagglutination was described by clustering process of colloidal particles by measuring the surface potential of Tol 5 cells with and without AtaA nanofibers.

**Conclusions**

By the CLSM, Tol 5 wild-type (WT) but not  $\Delta ataA$  mutant was revealed to form a thick cell layer ( $> 30 \mu\text{m}$ ) with void structure on a glass surface. The process of the cell clumping was described by DLVO theory and CCA model, which were developed as colloidal aggregation models. The autoagglutination of Tol 5 cells was realized by Brownian motion and the collision of AtaA fibers, but not that of cell bodies. By flow cell analyses, it was demonstrated that autoagglutinated WT cells attached well to a glass surface, whereas its single cells hardly attached. These results suggest that autoagglutination greatly contributes to cell adhesion due to enlarged particle size.



**FEMS-1169**

**Biofilms**

**TRANSCRIPTION FACTORS IN CANDIDA ALBICANS BIOFILM FORMATION**

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**Background**

*Candida albicans* is a major fungal pathogen of humans. It is a commensal on the skin and mucosal surfaces, but can cause invasive and systemic infections particularly in the immunocompromised patients. *C. albicans* can easily form biofilm on supporting surfaces, such as mucosal surfaces and indwelling medical devices. Biofilms enhance *C. albicans* resistance to antifungals and may help cells to escape from the host immune systems.

**Objectives**

The identification and study of transcription factors related to biofilm formation can provide important insights into molecular mechanisms controlling biofilm formation.

**Methods**

The systems biology approach is a novel trend in biological research that focuses on the complex interactions among genes, proteins, and intracellular metabolites. Using systems biological approach, we identified several transcription factors that may involve in biofilm formation. *MSS11*-deleted mutant showed a defect in forming a mature biofilm and partially attenuates the virulence of *C. albicans* in a mouse model of infection. In contrast, deletion of the gene encoding another transcription factor enhanced cell adhesion and biofilm formation compared to wild type. Interestingly, this mutant also showed an increase in the expression of adhesin genes.

**Conclusions**

Taken together, our results support applying the systems biology approaches to study various aspects of fungal biofilm formation and pathogenesis.

**FEMS-1359**

**Biofilms**

**INTERFERENCE OF BIOFILM FORMATION BY LIPOPOLYSACCHARIDE O-ANTIGEN AND THE CAPSULE-LIKE COMPLEX OF FRANCISELLA TULARENSIS**

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**Background**

*Francisella novicida* forms a robust biofilm on most surfaces, whereas *F. tularensis* subsp. *tularensis* and subsp. *holarctica* form little or only a poor biofilm. *F. novicida* colonies are also dark red on Congo Red agar, whereas *F. tularensis* colonies are light salmon. However, colonies of *F. tularensis* lipopolysaccharide O-antigen mutants are also dark red in color. We hypothesized that the O-antigen, or other surface components, of *F. tularensis* interferes with biofilm formation.

**Objectives**

Our objectives were to determine if mutants of *F. tularensis* lacking O-antigen and/or a capsule-like complex (CLC) were more capable of forming a robust biofilm than parent strains.

**Methods**

*F. novicida*, *F. tularensis* (types A and B), and mutants were grown in polystyrene wells or on glass coverslips, and biofilms examined by crystal violet staining, confocal laser scanning microscopy, and scanning electron microscopy. Biofilm-associated exopolysaccharide (EPS) was isolated by enzyme digestion and phenol extraction, and composition determined by gas chromatography-mass spectrometry.

**Conclusions**

O-antigen mutants of *F. tularensis* made a more robust biofilm than parent strains within 10 days incubation. However, only a mutant lacking both O-antigen and CLC attached better and made significantly more biofilm than the parent after 15 days incubation. Some *F. novicida* O-antigen mutants also formed significantly more biofilm than the parent. Optimum biofilm formation was dependent upon the growth medium. A novel EPS was isolated and identified as glucan from the *F. tularensis* biofilm. We conclude that the O-antigen and CLC interfere with *F. tularensis* biofilm formation, likely through interfering with attachment.

**FEMS-2977**

**Biofilms**

### **3D VISUALIZATION AND QUANTIFICATION OF BIOFILM IN POROUS MEDIA BY X-RAY TOMOGRAPHY**

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#### **Background**

The development of reliable models of biofilm growth in biofilters at the industrial scale, suffers from a lack of 3D measurements at the scale of the biofilter's constituents. Currently, only three experimental studies, using Synchrotron X-ray Microtomography, proposed protocols based on the use of different contrast agents to image such complex media at the pore scale.

#### **Objectives**

The aims of the present study, based on the adaptation of these protocols to laboratory X-ray microtomograph, are threefold:

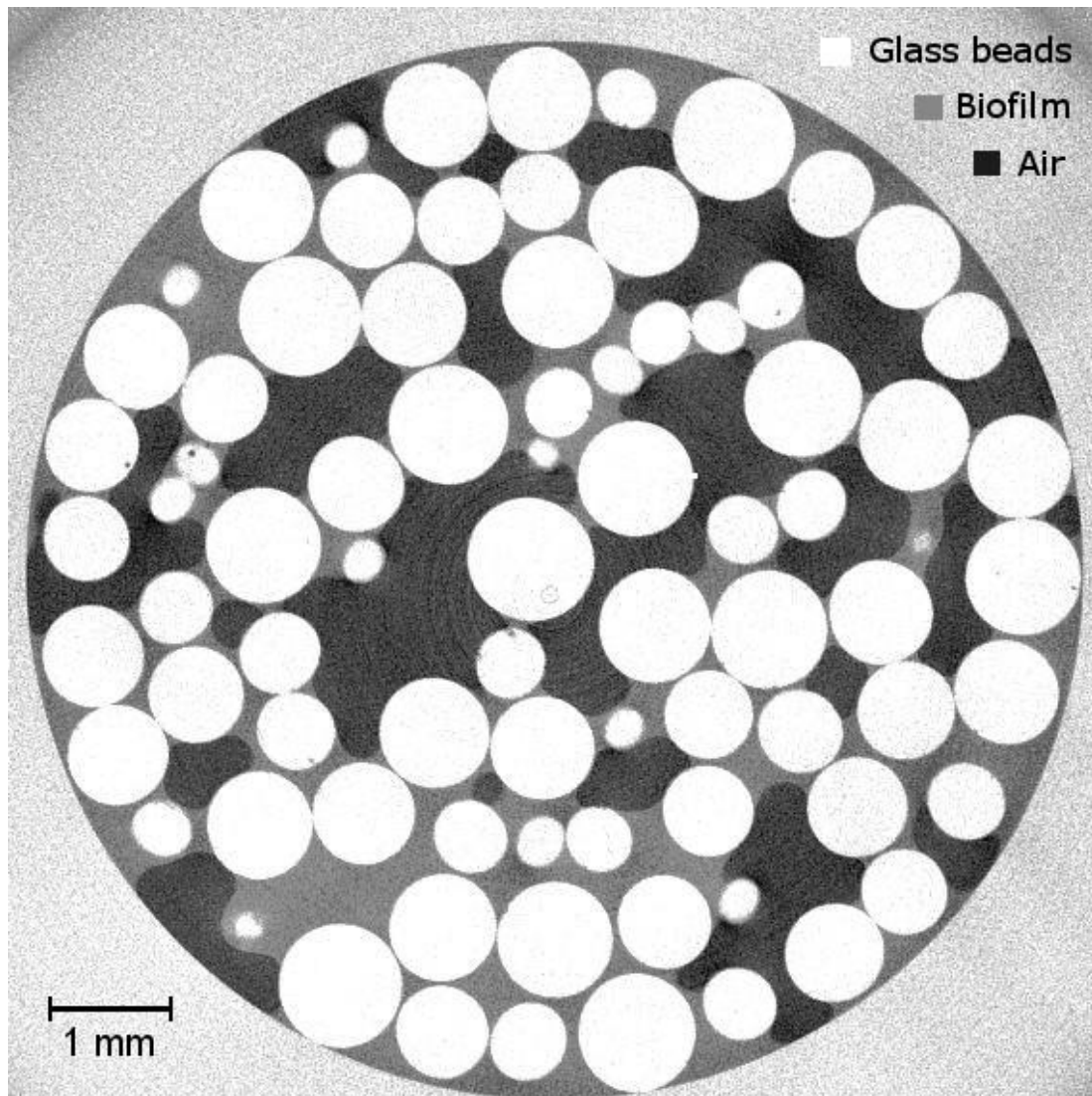
- (i) the 3D visualization and quantification of the interface between the biofilm and the bulk fluid. This will give us access to the shape, the spatial distribution and volume of the biofilm at the pore scale ;
- (ii) the numerical evaluation of physical properties such as permeability or diffusivity arising in macroscopic models using up-scaling methods ;
- (iii) the evolution of the aforementioned biofilm characteristics with time and according to the operating conditions (flow rate, nutrient conditions.)

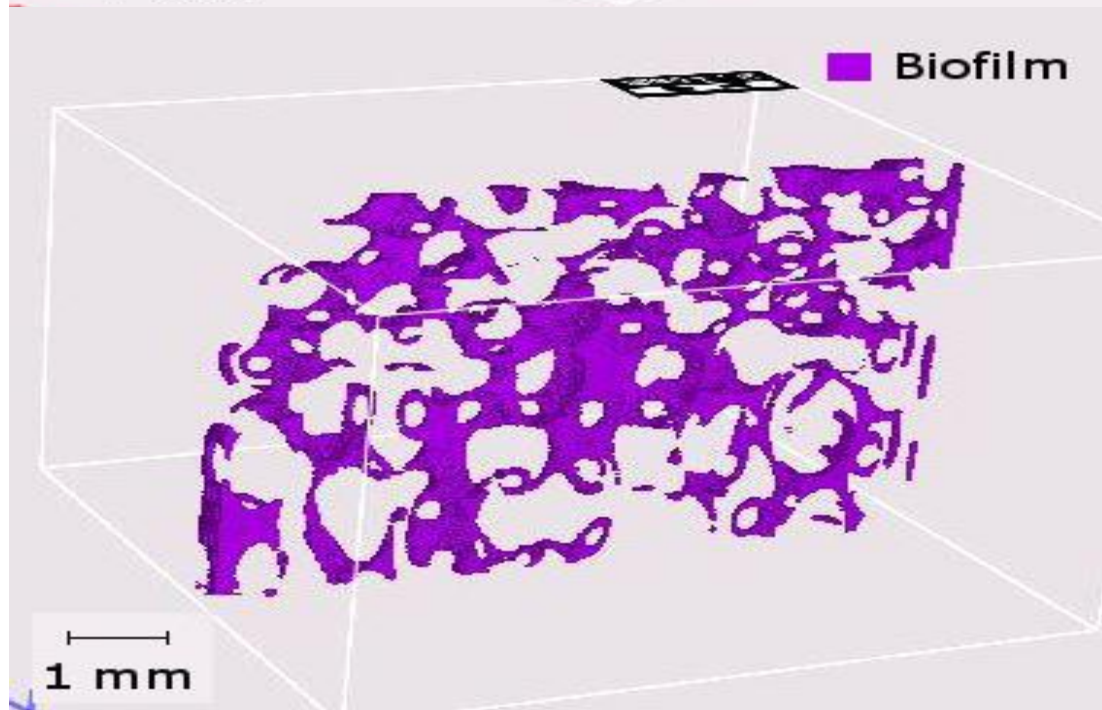
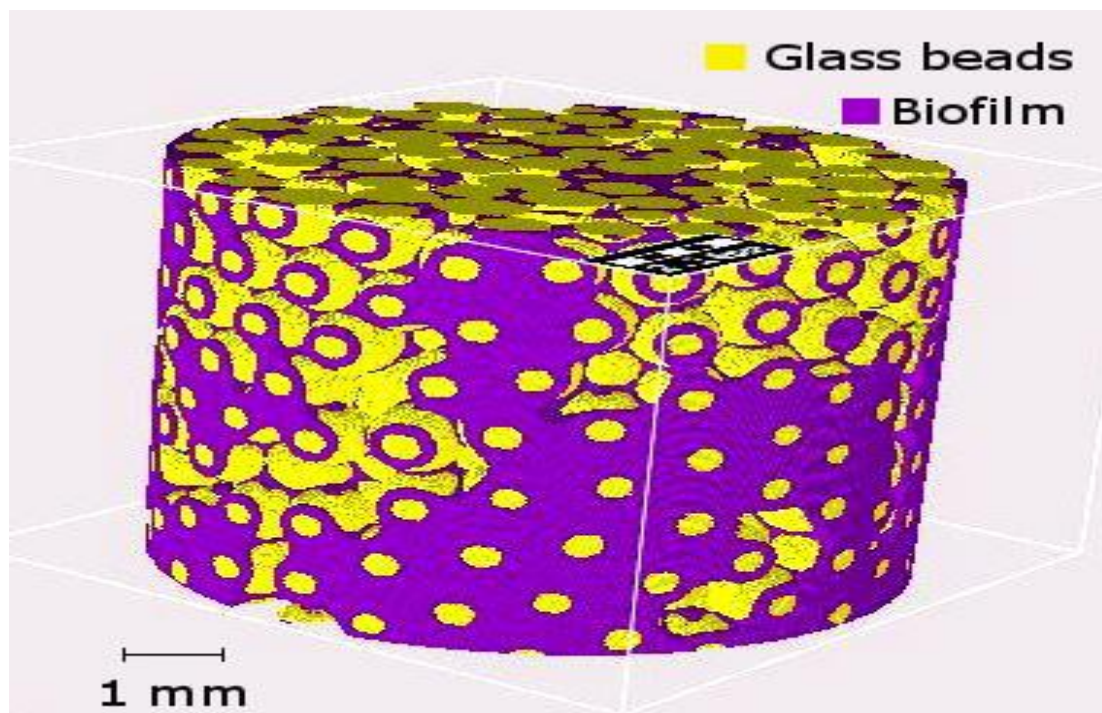
#### **Methods**

Thus, the *Pseudomonas putida* biofilms were grown on glass beads in small biofiltration columns. A new protocol permitting to avoid the use of contrast agent was proposed and 3D X-ray images of the biofilms grown under various operating conditions were obtained (i,iii).

#### **Conclusions**

The 3D microstructural properties were quantified using appropriated image processing tools (i) and the physical properties were estimated using numerical tools (ii). The representativity of the imaged volume was checked for both structural and physical properties giving access to some of the missing elements to build the macroscopic model.





**FEMS-1013**

**Biofilms**

**ANTI-BIOFILM FORMING EFFECT OF ESSENTIAL OILS ON LISTERIA MONOCYTOGENES IN MONO- AND MIXED CULTURES**

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**Background**

*Listeria monocytogenes* can be found frequently in mono- or mixed culture biofilms. These make sanitization difficult and can lead to cross-contamination and food safety problems. Beside heat treatment, preservatives can be used to eliminate pathogens but sometimes they are not effective and can produce unpleasant by-products. Application of natural antimicrobial agents like essential oils (EOs) could be a potential solution.

**Objectives**

Our aim was to investigate the anti-biofilm forming effect of selected EOs and their major components on *L. monocytogenes* biofilms and on mixed-cultures of *L. monocytogenes* and *E. coli*.

**Methods**

MIC values were determined by micro-dilution method. Biofilm formation was conducted in microtiter plates; after 4 h adhesion time, fresh medium containing EOs or components was added in MIC/2 concentration. Biofilm formation was monitored by crystal violet staining. For mixed cultures *L. monocytogenes* and *E. coli* were mixed in 1:1 ratio and concentration intervals between the MIC values of the two bacteria were used.

**Conclusions**

In case of monocultures, cinnamon and cinnamaldehyde have the best anti-biofilm formation effect. For mixed cultures, the effect of the EOs was concentration dependent:  $\alpha$ -pinene and linalool showed better anti-biofilm forming effect than the parent essential oil.



**FEMS-2114**

**Biofilms**

**CHARACTERIZATION OF NaCl STRESS-INDUCIBLE PALMELLOIDS AND UNDERSTANDING THE ROLE OF A STRESS-RESPONSIVE GENE, *BOLA* FROM THE GREEN CHLOROPHYTE *CHLAMYDOMONAS REINHARDTII*.**

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**Background**

*Chlamydomonas reinhardtii*, a unicellular green alga, is a preferred model organism for studying stress-induced responses. Depending on the stress dose and time of exposure, the flagella of this organism are paralyzed / lost and later cells manifest 'palmelloids' or apoptose. Palmelloids, akin to bacterial biofilms, are a stress-responsive temporary, dormant 'colonial' stage exhibiting conserved physiological changes.

**Objectives**

The current study deals with characterization of palmelloids formed by *C. reinhardtii* in response to NaCl stress and explores the role of a conserved stress-responsive gene family, *C. reinhardtii bolA* in biofilm / palmelloid formation.

**Methods**

A detailed morphological and biochemical analysis of NaCl stress inducible palmelloids was performed.

Drawing an analogy, it is known that the morphogene, *bolA*, significantly facilitates the formation of biofilms and induces morphological changes in response to stress in *Escherichia coli*. *Chlamydomonas reinhardtii* genome harbors five *bolA-like* genes. The ORF with highest homology to algal systems (*CrboLA-4*) was cloned and the protein over-expressed in *Escherichia coli*.

**Conclusions**

Palmelloids rapidly dissociated upon de-stress suggesting the reversibility of this phenomenon. The cells in palmelloids displayed conserved physiological features such as clustering of cells in a common envelope, decreased viability, accumulation of lipid and starch granules and presence of extracellular polysaccharides. An iTRAQ analysis of the spent medium from palmelloid-containing cells suggests a strong involvement of cell wall proteins. Further heterologous over-expression of *CrboLA-4* in *E. coli* did not affect its growth; but, induced biofilm formation and changed its morphology, indicating functional conservancy. Its role in the NaCl-induced palmelloidy remains to be explored.





**FEMS-0930**

**Biofilms**

**OLSBA OPERON INVOLVED IN ORNITHINE LIPID BIOSYNTHESIS AFFECTS VIRULENCE AND BIOFILM FORMATION OF PSEUDOMONAS AERUGINOSA**

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**Background**

Ornithine lipids (OLs) are interesting bacterial lipids that are widely found in outer membrane of many Gram-negative bacteria, but not detected in Eukarya and Archaea. *Pseudomonas aeruginosa* has *olsBA* operon encoding acyltransferases that functions the OL biosynthesis. OlsBA works in two steps for the OL biosynthesis, in which OlsB transfers an acyl group to ornithine to make lyso-ornithine lipid and OlsA converts the lyso-ornithine lipid into ornithine lipid by another acyl-group transfer.

**Objectives**

Acyltransferase genes of *P. aeruginosa* were originally screened for the effect on the virulence and biofilm formation of *P. aeruginosa*. The *olsBA* operon was found to have significant pleiotropic effects on the virulence related-phenotypes of *P. aeruginosa*. In this study, we addressed how OlsBA modulates the virulence and biofilm formation of *P. aeruginosa*.

**Methods**

1) The virulence was investigated by using two different host models, *Tenebrio molitor*, an insect and *Caenorhabditis elegans*, a nematode. 2) The effect of the *olsBA* overexpression on biofilm formation in *P. aeruginosa* was analyzed in flow cell system. 3) The host response to OLs was monitored through the expressions of COX-2 and iNOS using Western blot and real-time PCR analyses.

**Conclusions**

We found that the overexpression of *olsBA* operon modulated some virulence related-phenotypes of *P. aeruginosa* by reducing the quorum sensing response. In addition, the overproduced OLs directly alleviate the virulence of *P. aeruginosa*, enhances biofilm formation, and modulates the host physiology.

FEMS-1387

Biofilms

## THE NITROGEN DIOXIDE INCREASES PSEUDOMONAS FLUORESCENS BIOFILM FORMATION: IDENTIFICATION OF THE BACTERIAL RESPONSE TO AIR POLLUTANT

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### Background

Nitrogen dioxide is an air pollutant of increasing interest in biology. Exposure of animals to NO<sub>2</sub> results in several toxic effects, mostly lung injury.

### Objectives

Aware of the effect of NO<sub>2</sub> on pseudomonal biofilm formation, we looked for the mechanisms of bacterial response that could explain their resistance to NO<sub>2</sub>.

### Methods

The confocal microscopy was used for biofilm studying, completed by motility assays. In a second time, *in silico* and *in vitro* genomics tools were used to narrow our research scope. Additionally, in order to characterize the mechanism of *P. fluorescens* resistance to NO<sub>2</sub> pollution these data were completed with proteomics and lipidomics studies. For this purpose, the MALDI-TOF MS Imaging was coupled to HPTLC and compiled with the traditional GC-MS.

### Conclusions

When the NO<sub>2</sub> increases *P. fluorescens* biofilm formation, the bacterial motility decreases. In coherence, the level of cyclic di-GMP evolves in NO<sub>2</sub> exposed cells. The lipidic study shows no drastic change in membrane charge and its composition in phospholipids and fatty acids. This suggests that NO<sub>2</sub> free radical could freely pass through membrane. In contrast, NO<sub>2</sub> promotes an extensive modification in protein production, notably the over-production of the proteins of stress response, involved in oxidative stress tolerance and iron transport/metabolism. Thus both iron up-regulation and C-di-GMP level could be signals for biofilm development. This knowledge should probably offer therapeutical solutions in antibacterial treatment.

**FEMS-0719**

**Biofilms**

## **OXYGEN LIMITATION TRIGGERS PELLICLE FORMATION IN BURKHOLDERIA GLUMAE**

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### **Background**

Bacteria adapt to unfavorable environmental conditions for survival. *Burkholderia glumae*, the causal agent of rice panicle blight, absolutely requires oxygen for respiration. For aerobes such as *B. glumae*, oxygen limitation is a serious problem for survival. Many bacteria including *B. glumae* possess genes encoding proteins with a PAS/PAC domain whose functions sense changing environments and manage gene regulation accordingly. Oxygen availability in bacteria is related to biofilm or pellicle formation. Cyclic-di-GMP synthesized by proteins carrying a GGDEF domain is a messenger for the pellicle formation.

### **Objectives**

We aimed to investigate how *B. glumae* adapts to oxygen availability

### **Methods**

Overnight culture of *B. glumae* grown in LB broth was serially diluted with the same medium to give approximately  $1 \times 10^7$  cells/ml. A portion of 200  $\mu$ l was placed in 96 well cell culture plates and incubated at 37°C without shaking. Growth was monitored from day 0 to day 7 of the incubation period. Morphologically altered colonies were isolated, and a mutation responsible for colony variation was identified by whole genome re-sequencing.

### **Conclusions**

Mutations in the *bpaA* (*B. glumae* pH associated protein A) gene encoding a protein possessing PAS/PAC and phosphatase domains conferred wrinkly colony types. A mutation in *bpaA* resulted in elevated expression of cellulose biosynthesis genes responsible for pellicle formation. Thus, mutations in *bpaA* and pellicle formation in oxygen limited conditions suggest how *B. glumae* has evolved to survive under unfavorable conditions.

**FEMS-1168**

**Biofilms**

**ROLE OF EACH EPM GENE ON THE FORMATION OF A NOVEL BIOFILM-  
SCAFFOLD EXTRACELLULAR POLYMER FROM PSEUDOMONAS**

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**Background**

*Pseudomonas alkylphenolia* KL28 forms unique three-dimensional biofilm bodies depending on the environmental stimuli. It forms highly branched mushroom-like aerial architecture during vapor *p*-cresol utilization and circular floating pellicles on standing LB medium. These indicate that *P. alkylphenolia* is a good bacterial sample for studying bacterial community formation and cell-cell interactions. An *epm* gene cluster consisting of eleven genes showing deduced amino acid sequence similarities to those encoded on *alg* genes has been identified to be responsible for formation of the special biofilm structures.

**Objectives**

In this study, the role of each *epm* gene was studied in relation to the biofilm formation and polymer processing.

**Methods**

For this purpose, mutants specifically deleted in *epm* genes from an *epm*-overproducing spontaneous mutant SG1-WC1-10' were constructed. With those mutants, changes in surface-related phenotypes and polymer production were examined.

**Conclusions**

It was found that *epm* genes except *epmL* are required to form wrinkle colonies and mature biofilms including aerial architecture and circular floating bodies. Mutants in *epmI*, *epmJ*, *epmX* (acetylation, deduced from *alg* genes), *epmG* (epimerization), *epmE*, *epmK* (transporter) were still positive to polyuronic acid production. In addition, some of these *epm* genes cannot be substituted by *alg* genes. Thus, these results indicate that regardless of the similarities between Alg and Epm proteins the structure of Epm polymer derived from an *epm* gene cluster is different from alginate and the process of Epm polymer biosynthesis is different from that of alginate in *Pseudomonas*.



**FEMS-0902**

**Biofilms**

## **INDOLE EFFECT ON ANTHRANILATE DEGRADATION IN PSEUDOMONAS AERUGINOSA**

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### **Background**

Anthranilate and indole are alternative degradation products of tryptophan in nature. *Pseudomonas aeruginosa* produces anthranilate from tryptophan degradation. Previous studies showed that anthranilate and indole has an opposite effect on *P. aeruginosa* biofilm formation. While indole enhances the biofilm formation of *P. aeruginosa*, anthranilate was able to disintegrate the mushroom structure of biofilm.

### **Objectives**

We addressed how indole could enhance the biofilm formation of *P. aeruginosa* in relation to anthranilate degradation.

### **Methods**

*P. aeruginosa* biofilm formation was analyzed in flow-cell system. High Performance Liquid Chromatography was used to measure the level of anthranilate in *P. aeruginosa* culture supernatant. The effect of indole on AntR activity was analyzed by reporter fusion *in vivo* and gel shift assay *in vitro*.

### **Conclusions**

In *P. aeruginosa*, the transcription of *antABC* operon encoding anthranilate dioxygenase complex that functions to degrade anthranilate, was activated by the indole treatment. Although indole alone failed to activate AntR, co-addition of indole with anthranilate boosted the activation of AntR by anthranilate. As consequence, the anthranilate level in *P. aeruginosa* culture supernatant decreased. Since anthranilate has a biofilm-disintegrating effect, we suggest that indole may enhance the biofilm formation of *P. aeruginosa* by reducing anthranilate level through the modulation of AntR activity.

**FEMS-0957**

**Biofilms**

**TRIMERIC AUTOTRANSPORTER ADHESINS (TAAS) - STRUCTURAL STUDIES OF THE TRANSLOCATION PROCESS, AND PERSPECTIVES FOR ITS INHIBITION**

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**Background**

Trimeric autotransporter adhesins (TAAs) comprise a widespread family of adhesive molecules in Gram-negative bacteria, many of which are important virulence factors [1]. The prototypical trimeric autotransporter is the *Yersinia* adhesin YadA from *Yersinia enterocolitica*, which mediates attachment to collagen and other extracellular matrix (ECM) molecules, promotes serum resistance and mediates autoagglutination.

**Objectives**

The autotransport process depends on a conserved translocation domain and associated factors. Inhibiting this process is a possible antimicrobial strategy, as no homologous systems exist in Eukaryotes, and as many of the TAAs are important or even essential in host colonization by a broad range of Gram-negative pathogens.

**Methods**

We recently solved the structure of the *Yersinia* YadA translocation domain (using solid-state NMR [2]) and of a chaperone-like protein involved in the translocation of *Salmonella* SadA (using x-ray crystallography [3]). Conserved residues thought to be important for translocation are targeted by mutagenesis. Adhesion assays and antibody-based assays such as FACS are used to quantify translocation.

**Conclusions**

A better understanding of the translocation process on the molecular level is essential for efficient inhibition of the surface display of these important pathogenicity factors. The methods developed to quantify surface localization can be scaled up for systematic screens for antimicrobial substances.

[1] Leo *et al.*, Phil Trans Royal Soc B 2012

[2] Shahid *et al.*, Nature Methods 2012

[3] Leo *et al.*, JBC 2014



**FEMS-2206**

**Biofilms**

## **DISSECTING METHANO- AND METHYLOTROPHIC BIOFILM FUNCTIONING IN A IODINE-RICH SUBSURFACE SPRING CAVERN**

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### **Background**

Massive microbial biofilms have recently been discovered in a subsurface spring cavern fed by iodine-rich deep formation water with high thermogenic methane loading. The biomass produced by the microbes and exopolysaccharide slime completely covers the walls and ceilings of the cave, the latter bearing snottites of up to 10 cm length.

### **Objectives**

However, the nature of these unique biofilms as well as their ecophysiology are not yet understood.

### **Methods**

Our primary taxonomic characterization of the biofilms revealed a surprisingly diverse polymicrobial community assembly, substantiating a complex network of bacteria and archaea interacting within the sticky matrix. Distinct methanotrophic and methylotrophic populations within the *Alpha*-, *Beta*- and *Gammaproteobacteria* dominated the biofilms. *Methylophilaceae* and *Methylococcaceae* were abundant on the walls and especially in the cavern water, but not so in ceiling biofilms. Here, ribosomal and functional gene analysis hinted at dominating methylotrophic and potentially also iodine-cycling populations, thus supporting a hypothesized cycling of iodomethane in this system. Geochemical gradients in the cavern were clearly reflected within community assembly, as well as elemental and stable isotope composition of the biofilms. These findings were further supported by distinct microaerophilic oxidation rate measurements for methano- and methylotroph substrates in spatially resolved biofilm samples. Ongoing work involves the elucidation of ecological niche partitioning between strict methanotrophs and potential methyl halide-oxidising populations in this unique habitat.

### **Conclusions**

In conclusion, primary insights into a dominantly chemolithoautotrophic natural biofilm system are revealed, thriving on deep subsurface energy inputs but only a few meters under our feet.

**FEMS-0943**

**Biofilms**

## **EFFECT OF SUBINHIBITORY CONCENTRATIONS OF CEFOTAXIME ON BIOFILM FORMATION IN STREPTOCOCCUS PNEUMONIAE**

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### **Background**

*Streptococcus pneumoniae* forms biofilm to persist in the human nasopharynx. Biofilms are often associated with pneumococcal pneumonia and otitis media.  $\beta$ -lactam agents remain the first line antibiotic for the treatment of *S. pneumoniae* infections. Subinhibitory concentrations of antibiotics have been reported to enhance biofilm formation.

### **Objectives**

This study aims to evaluate the effect of subinhibitory concentrations of a third generation cephalosporin, cefotaxime (CTX) on biofilm formation in *S. pneumoniae*.

### **Methods**

The effect of CTX (at 1/2, 1/4 and 1/16 MIC) were tested against biofilm formation in R6 and four clinical isolates in 24-well microtiter plates. Crystal violet (CV) staining and colony forming unit (CFU) were used to quantify biofilm biomass. Similar experiments were carried out with erythromycin and ciprofloxacin.

CV staining results showed that 1/2 MIC CTX resulted in 52.3%, 64.9%, 68.2%, 69.5% and 84.5% (all  $p$  values  $<0.01$ ) reduction in biomass of biofilm for R6 and the clinical strains tested. 1/4 MIC CTX led to 13.2% to 57.5% ( $p$  value  $<0.01$ ) reduction of biofilm while the inhibitory effect of 1/16 MIC CTX on biofilm formation were insignificant ( $<20\%$  inhibition). Living bacterial cells of R6 and two cefotaxime resistant strains recovered from biofilms unexposed to CTX were  $1.5 \times 10^8$ ,  $3.6 \times 10^4$  and  $1.3 \times 10^4$  CFU/well, respectively and decreased to  $4.7 \times 10^7$ , 53 and 43 CFU/well, respectively when exposed to 1/2 MIC CTX. Subinhibitory concentrations of non- $\beta$ -lactam antibiotics showed no significant influence on biofilm formation.

### **Conclusions**

Subinhibitory concentrations of CTX significantly inhibited biofilm formation.

**FEMS-1967**

**Biofilms**

**CHARACTERIZATION OF A NAPHTHALENE-DEGRADING MULTI-SPECIES BIOFILM ISOLATED FROM OIL-CONTAMINATED GROUND WATER**

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**Background**

Environmental microorganisms exist predominantly as biofilms and gain high tolerance to environmental stresses. Biofilm systems are especially suitable for the treatment of recalcitrant compounds as polycyclic aromatic hydrocarbon (PAH) because of their high microbial biomass and ability to immobilize compounds. To our knowledge, reports on naphthalene degradation by biofilm-forming bacteria are limited. In this study we isolated multi-species biofilm degrading naphthalene from oil-contaminated ground water (oil refinery, Murcia, Spain).

**Objectives**

The aims of this work were to characterize the biofilm microbial community and the biofilm structure and to isolate the bacteria involved in the biofilm formation and degradation of naphthalene.

**Methods**

The microbial community composition was characterized by culture dependent and molecular methods. For a qualitative understanding of biofilm structure and composition we used Scanning Electron Microscope (SEM). We also designed degenerated probes to detect and amplify naphthalene dioxygenase *nahA* gene homologues from environmental DNA and bacterial isolates.

**Conclusions**

Molecular analyses showed that the initial community was dominated by Betaproteobacteria (79% *Rhodospirillum rubrum*, 6% *Azotobacter*). After enrichment on naphthalene minimal medium we found Betaproteobacteria (54% *Variovorax*) and Alphaproteobacteria (43% of a *Xanthobacteraceae* strain) as dominant bacteria. We isolated members of *Microbacterium*, *Starkeya*, *Rhizobium*, *Brevundimonas*, *Variovorax*, *Pseudomonas*, *Pseudoxanthomonas*, *Epilithonimonas* and *Aquabacter*. Some of the isolates degraded naphthalene in pure culture and some other were able to produce biofilm. Homologues of *nahA* genes could be retrieved from the enrichment cultures and from some isolates.

**FEMS-1984**

**Biofilms**

**NITRIC OXIDE (NO) DONORS TO DISPERSE BIOFILMS OF INDUSTRIAL SIGNIFICANCE FORMED BY SALMONELLA ENTERICA, PATHOGENIC ESCHERICHIA COLI AND LISTERIA SP.**

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<sup>2</sup>Soil and Water Science, University of Florida, Gainesville, USA

**Background**

Biofilms formed in post-harvest production facilities are recalcitrant reservoirs of pathogens, which are difficult to control. Pathogens in biofilms are resistant to common disinfectants and their mechanical removal is only partially effective. Therefore, novel approaches for controlling biofilms are needed. Recent discoveries of the function of nitric oxide in dispersing preformed biofilms offer an opportunity to test the feasibility of using this gas in industrial applications.

**Objectives**

Determine the effect of several nitric oxide donors on biofilm of *Salmonella enterica*, *Escherichia coli* and *Listeria innocua* preformed on surfaces of industrial interest.

**Methods**

Biofilms were pre-formed in appropriate media for 24 hours or 1 week in 96-well plastic plates (polypropylene, polystyrene). After incubation, medium was removed and nitric oxide donors were resuspended in Phosphate Saline Buffer (PBS) and added to the wells using different concentrations. Biofilms were exposed to Molsidomine and MAHMA-NONOate donors from 2 hours to 6 hours at 22°C and 4°C to mimic the post-harvest environments. Dispersal was measured by staining the remaining biofilms using crystal violet or using a GFP labeled *Salmonella* strain. The donors were also tested in association with a cellulose nanocrystals hydrogel (CNC).

**Conclusions**

Molsidomine and MAHMA-NONOate were able to disperse at least 50% of the biofilms preformed by human pathogens, *Salmonella enterica* 14028, *Escherichia coli* O157 (EHEC). The association of the two nitric oxide donors with cellulose nanocrystals was also effective in dispersing *Salmonella* preformed biofilms on polypropylene up to 15% of the total biomass. Our results show that nitric oxide donors expand the toolset of proactive solutions for removing industrial biofilms.



**FEMS-2799**

**Biofilms**

**Effect of pomegranate peel extract on biofilm and planktonic cells of *Candida albicans***

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Background: Pomegranate peel is used by many people against diarrhea, ulcers, periodontal disease, stomatitis and pharyngitis. *Candida albicans* can infect any part of the body (gum, tongue, toes...) with tissue invasion thus inducing candidiasis. *Candida* infections are difficult to manage due to their persistent nature and the Biofilm growth of this yeast serves as an increasing source of clinical infections.

Objectives : This study investigates the antioxidant activity of acetone peel extract of sour pomegranate peel named Quares (QE) and its effect against biofilm and planktonic cells of *Candida albicans*.

Methods: acetone 50% (v/v) was used for phenolic compounds extraction. The antifungal activity is evaluated using three strains of: CA1, CA2 and CA3. Various concentrations have been used to determine the MIC. Preliminary screening of activity: The agar disc diffusion method was conducted. The concentrations tested are 10mg/disc to 0.5mg/disc. Two models were used for the biofilm formation inhibition of *Candida albicans* on micoplaque (Hamada et al., 1978) and on hydroxyapatite discs (Guggenheim, 2001). The samples were then viewed under a scanning electron microscope.

Results and discussion: The extract from the variety QE showed a high content of total polyphenols. The inhibition percent of H<sub>2</sub>O<sub>2</sub> is 96.32 ± 0.56 %. The inhibition of *Candida albicans* biofilm formation on hydroxyapatite test showed strong inhibitory action against the strains in this order CA1 > CA3 > CA2.

Conclusion: This study reports the high content of phenolic compounds of pomegranate peel extract, antioxidant activity and *Candida albicans* biofilm inhibition.

**FEMS-2145**

**Biofilms**

**IDENTIFICATION OF CANDIDA SPECIES ISOLATED FROM MEDICAL DEVICES  
CONNECTED MEDICAL ICU PATIENT AND EVALUATION OF THEIR BIOFILM  
FORMATION AND DRUG SUSCEPTIBILITY**

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Background: Nowadays, mortality and morbidity due to infections caused by biofilm on medical devices such as catheter and implants are increasing and *Candida* has become one of the most common causes of nosocomial infections. One of the most important characteristics of biofilms is broad-spectrum resistance to antimicrobial drugs. However, too much using of this medicine can lead to the resistance of *Candida* species to fluconazole. Method: A total of 60 samples of medical devices attached to patients in ICU were investigated. Yeast colonies isolated from medical devices using routine laboratory procedures were identified. The MIC of fluconazole for all samples was carried out using Broth Microdilution according to CLSI. To assess biofilm formation by MTT, the experiment was carried out in a 96-cell microplate. Over half of the isolates of *Candida* had the ability to form biofilms, and the ability of biofilm formation was read by ELISA reader at a wavelength of 570 nm. Results: Out of 60 samples, 48 isolates of *Candida* with an abundance of *Candida albicans* (42%), *C. glabrata* (27%), *C. kruise* (17%) and *C. tropicalis* (14%) were identified. Among the isolates, 9 were susceptible to fluconazole (19%), 10 cases were dose dependent (21%) and 29 were resistant to fluconazole (60%). *Candida* isolates had the ability to form biofilms. *C. albicans* and *C. tropicalis* had the maximum and minimum power of biofilm formation respectively. Conclusions: The results showed that *Candida* was resistant to fluconazole. All resistant isolates had the ability to form biofilms.



**FEMS-2915**

**Biofilms**

**MICROFLUIDIC MODEL FOR THE INVESTIGATION OF PSEUDOMONAS  
AERUGINOSA BIOFILMS**

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<sup>1</sup>*Biological Engineering, University of Guelph, Guelph, Canada*

**Background**

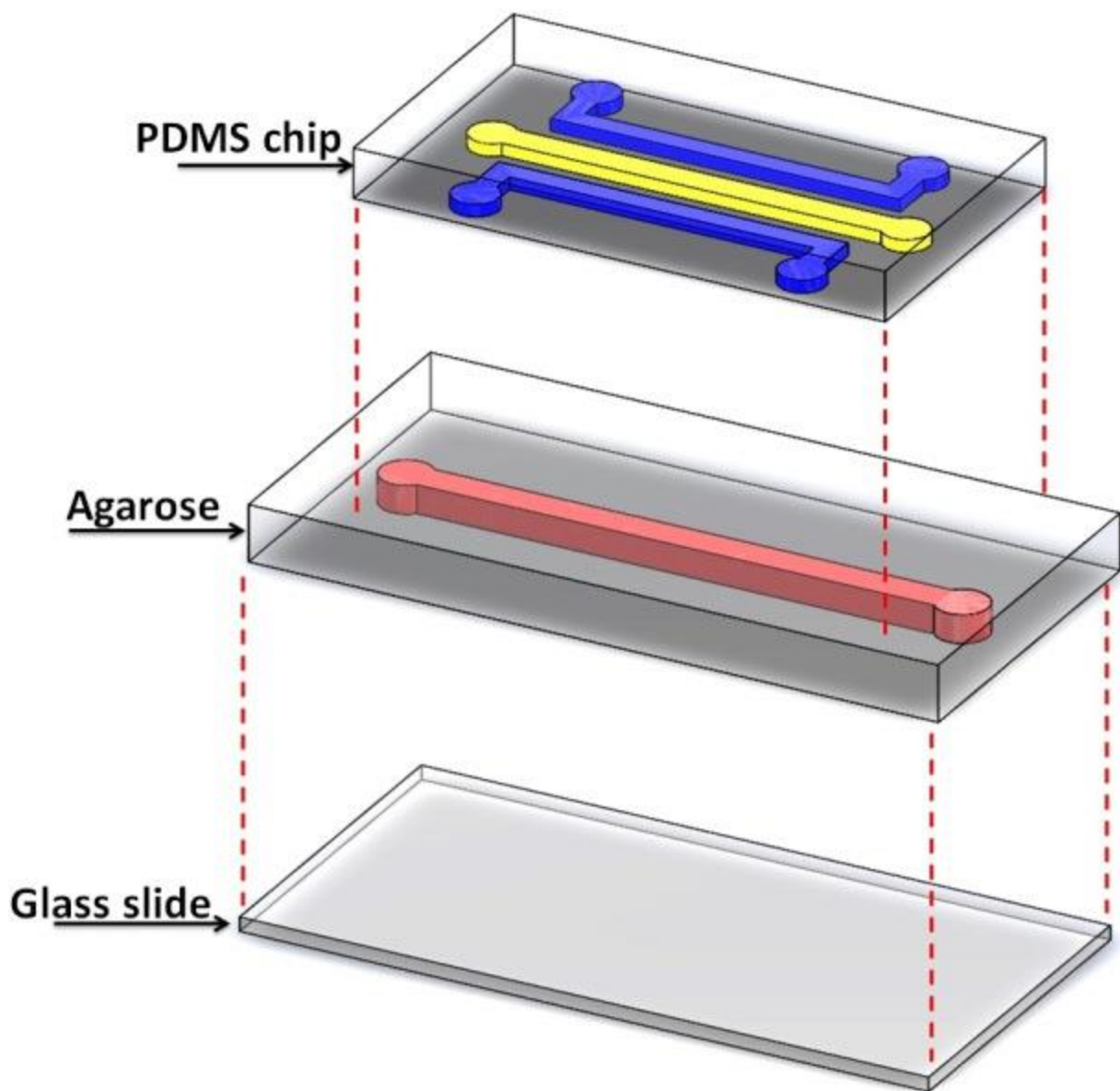
*Pseudomonas aeruginosa* is an opportunistic pathogen due to its capacity to form recalcitrant biofilm structures, while cohabiting with other harmful/pathogenic species and harboring the capability to release toxins that cause tissue necrosis. *P. aeruginosa* has been implicated in a number of microbially-induced or aggravated diseases, including cystic fibrosis and wound infections (e.g. burns, bites). Very little is known about the complex interactions that occur within polymicrobial communities and few tools exist for studying these interactions.

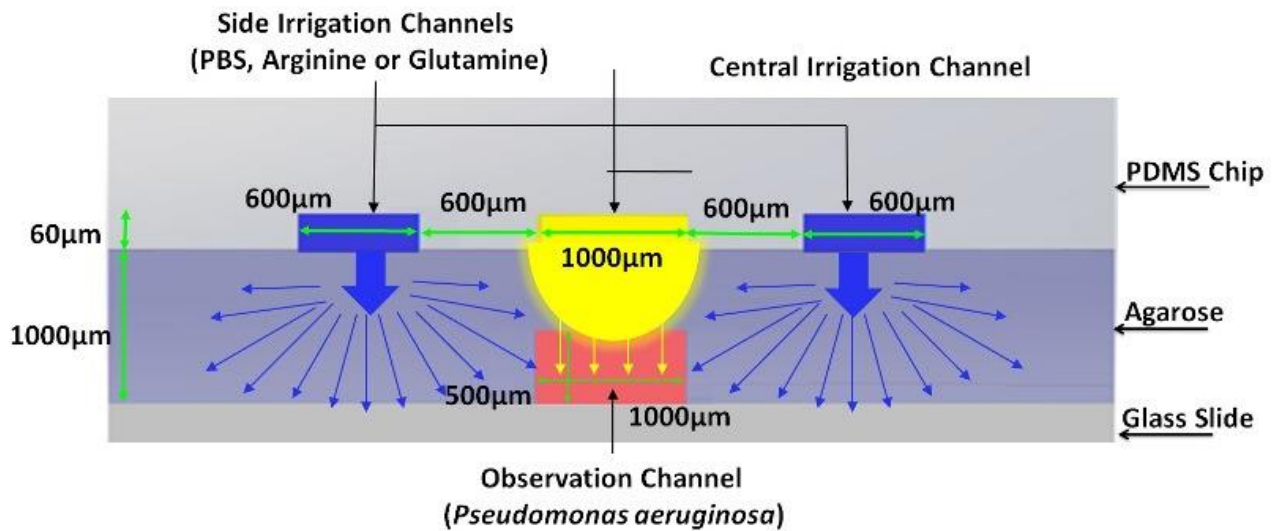
**Objectives**

In this study, we report on the development of a microfluidic model that mimics the relevant physiological properties of the wound microenvironment while incorporating materials present in the human extracellular matrix/wound environment.

**Methods**

We have validated the robustness of our model comparing traditional GFP-tagging to new fluorescent staining techniques to visualize/resolve individual species within a polymicrobial habitat. We have also demonstrated that chemotactic stimuli may be incorporated into our model through specialized ports in our chamber; we can readily monitor changes in motility in response to the introduction of amino acids, such as arginine and glutamine.

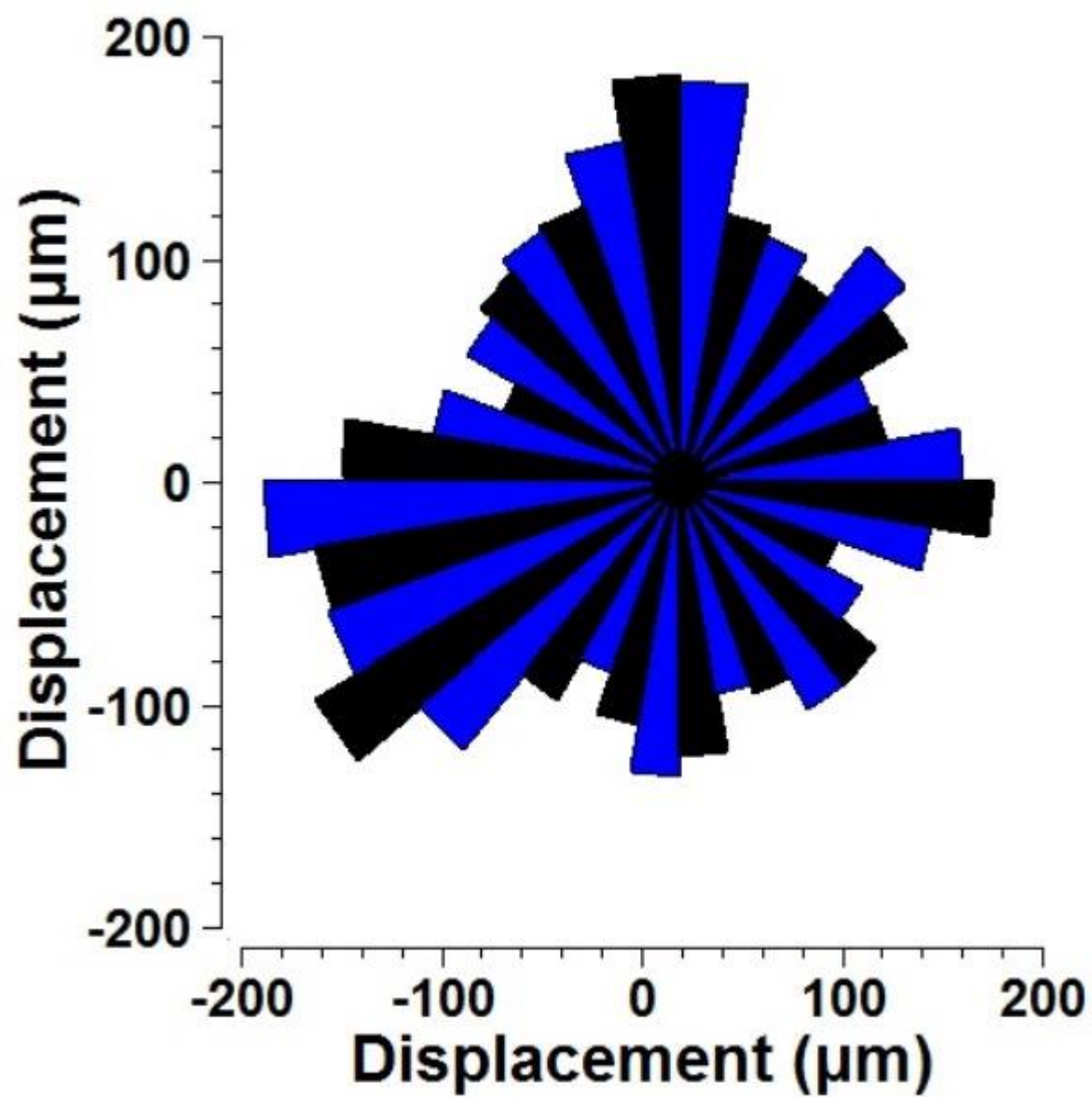




## Conclusions

We show that our model can be used to investigate the spatio-temporal mechanobiological structures of the wound environment, which will significantly contribute to our understanding of the development and progression of polymicrobial biofilm infections and its interactions with the host environment. Through the implementation of microfluidic platforms, we pose complex and relevant questions that may enhance our understanding of microbial interactions, all with the goal of

identifying new and specific targets for more effective antimicrobial therapies.



**FEMS-2749**

**Biofilms**

**IDENTIFICATION OF BIOFILM-RELATED GENES OF LISTERIA  
MONOCYTOGENES ISOLATED FROM SEAFOOD PROCESSING PREMISES**

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New Zealand

**Background**

*Listeria monocytogenes* is a foodborne human pathogen which is commonly found in the environment and is capable of withstanding environmental threats by adhering to surfaces to form a biofilm. Biofilm formation is believed to be a major factor for cross-contamination in food processing premises and therefore, elucidating the molecular mechanism of biofilm formation in *L. monocytogenes* can contribute to secure food safety.

**Objectives**

To identify genes involved in biofilm formation of *L. monocytogenes* 15G01

**Methods**

A library of 6,500 mutants was constructed via transposon mutagenesis of *L. monocytogenes* 15G01, a persistent strain in New Zealand seafood processing premises, with the Himar1 mariner-based transposon. This library was screened for altered biofilm formation using a microtiter plate assay, using conditions defined as optimum through preliminary studies. Quantification of biofilm mass was undertaken by optical density measurement after staining with crystal violet. Mutants of interest were selected through statistical analysis (two-sample t-test,  $p \leq 0.05$ ). The insertion site of the transposon was identified through semi-arbitrary PCR followed by DNA sequencing or by genome sequencing, and the inactivated loci analysed using BLAST.

**Conclusions**

Sequencing results revealed genes previously known to be involved in biofilm formation by *Listeria monocytogenes* and/or other bacterial species, which corroborates the method of transposon mutagenesis for functional analysis of biofilm formation. Furthermore, in this study, new genes influencing biofilm formation (either positively or negatively) have been identified. Further functional analysis of these genes is ongoing to elucidate their roles in biofilm formation.

**FEMS-2978**

**Biofilms**

**HYPOCHLORITE-INDUCED OXIDATIVE STRESS STIMULATES C-DI-GMP SYNTHESIS AND BIOFILM FORMATION IN PSEUDOMONAS AERUGINOSA**

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**Background**

The opportunistic human pathogen *Pseudomonas aeruginosa* is able to adapt to a variety of often harmful environmental conditions due to different survival strategies including the formation of resistant biofilms.

**Objectives**

In this study, we investigated the adaptation of *P. aeruginosa* towards hypochlorite (HOCl), a strong oxidant used by human neutrophils to kill invading bacteria and which can be found in chronically inflamed host tissue, e.g. in the lungs of CF patients

**Methods**

In static biofilm assays, we observed a significant increase of up to 3-fold in initial cell attachment in the presence of sub-lethal hypochlorite concentrations. Transcriptome analyses revealed a substantial upregulation of genes involved in oxidative stress response as well as biofilm formation in the presence of hypochlorite. Among others, we identified a 26-fold upregulation of ORF PA3177 coding for a putative diguanylate-cyclase (DGC), which catalyzes the synthesis of the second messenger c-di-GMP, and therefore influences motility, biofilm formation and persistence in *P. aeruginosa*. Subsequent LC-MS/MS analyses revealed a strong increase in c-di-GMP levels suggesting a key role of this second messenger in hypochlorite induced adhesion and biofilm development. The DGC PA3177 was further characterized in more detail demonstrating its involvement in motility, biofilm formation, antimicrobial resistance and persistence in *P. aeruginosa*. Using a subset of different mutant strains, we were able to show that both the pel and psl exopolysaccharides are effectors in the PA3177-dependent c-di-GMP network.

**Conclusions**

Our results demonstrate that host-derived antimicrobials are sensed by invading bacteria and exert a huge impact on bacterial pathogenesis including biofilm formation.



## FEMS-1304

### Biofilms

#### **DISTINCT SAGA FROM HOSPITAL-ASSOCIATED CLADE A1 ENTEROCOCCUS FAECIUM STRAINS CONTRIBUTES TO BIOFILM FORMATION**

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### **Background**

*Enterococcus faecium* is an important nosocomial pathogen causing biofilm-mediated infections. Elucidating *E. faecium* biofilm pathogenesis is pivotal for development of new strategies to treat these infections. In several bacteria extracellular DNA (eDNA) and proteins act as matrix components contributing to biofilm development.

### **Objectives**

In this study, we investigated biofilm formation capacity and the role of eDNA and secreted proteins in 83 *E. faecium* strains with different phylogenetic origin that clustered in clade A1 and clade B.

### **Methods**

Although there was no significant difference in biofilm formation between *E. faecium* strains from both clades, addition of DNase I or proteinase K to biofilms demonstrated that eDNA is essential for biofilm formation in most *E. faecium* strains, while proteolysis primarily impacted on biofilm formation of hospital-associated *E. faecium* strains that cluster in clade A1. Secreted antigen A (SagA) was the most abundant protein in biofilms from clade A1 and B *E. faecium* strains, although localization differed between the two groups.

### **Conclusions**

*sagA* is present in all sequenced *E. faecium* strains, with a consistent difference in the repeat region between the clades, which correlated with proteinase K susceptibility in biofilms. This indicates an association between the SagA repeat profile and the localization and contribution of SagA in *E. faecium* biofilms.



**FEMS-1837**

**Biofilms**

**WCAJ DELETION ENHANCES BIOFILM FORMATION IN KLEBSIELLA PNEUMONIAE**

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<sup>1</sup>*Biotechnology, Indian Institute of Technology Kharagpur, Kharagpur, India*

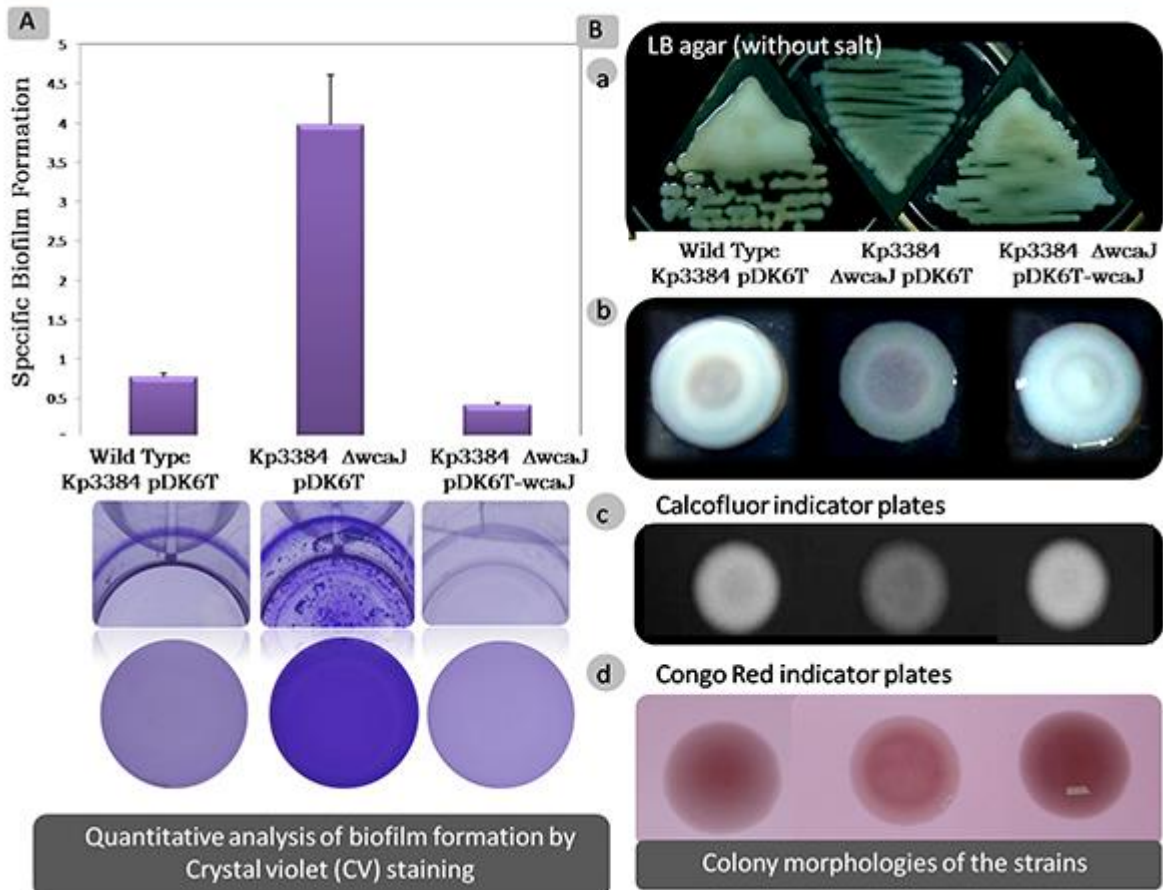
**Background**

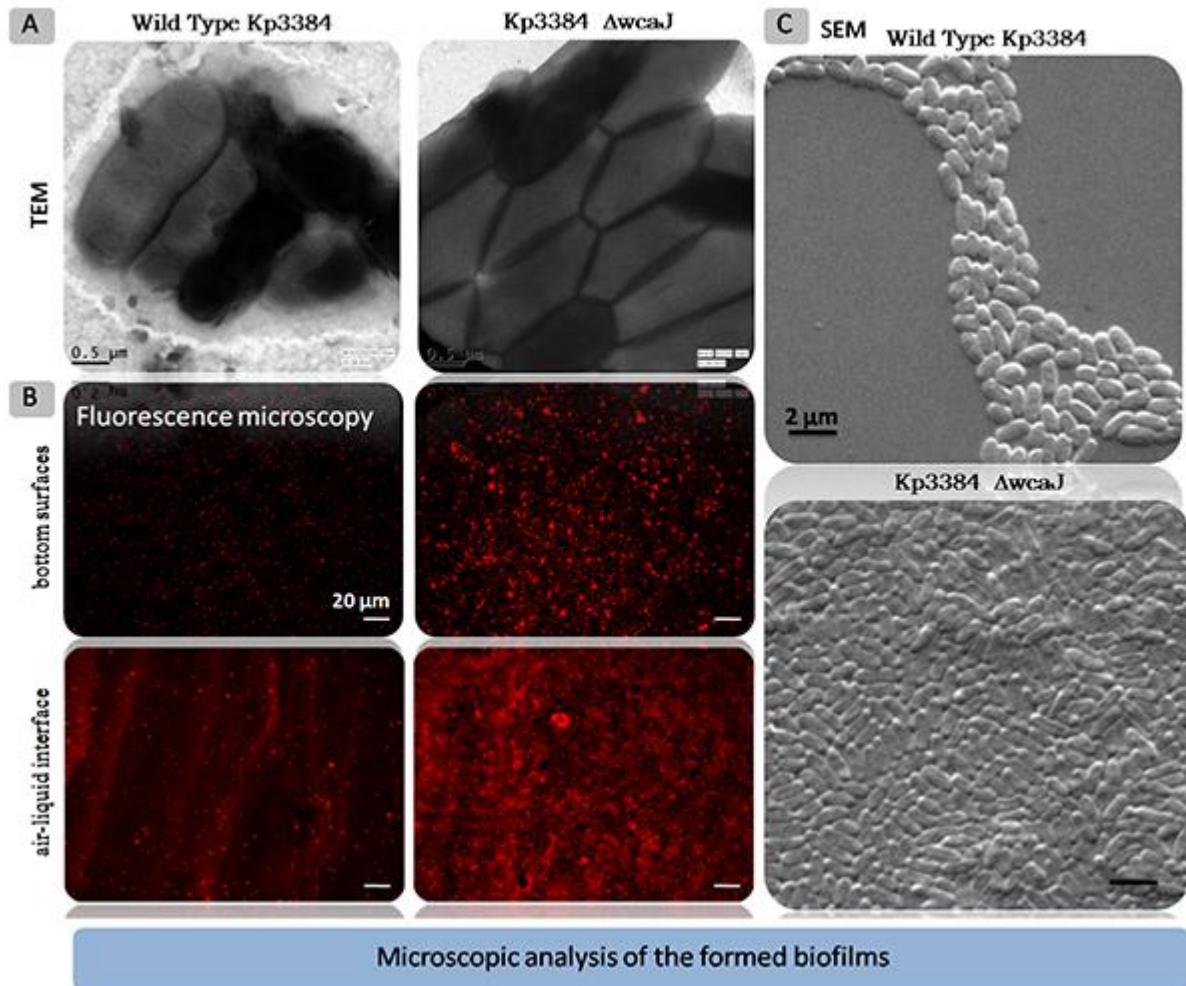
The worldwide occurrence and recent rapid spread of multiresistant strains of *Klebsiella pneumoniae* is of great concern and has generated an urgent need to identify new drug targets. Bacterial adhesion and biofilm formation are important for the establishment of *K. pneumoniae* infections. Elucidation of mechanism of biofilm formation by pathogens is the first step towards the development of novel approaches for the treatment and prevention of biofilm related infections.

**Objectives**

The goal of this study was to gain additional insights into biofilm formation in *K. pneumoniae* MTCC 3384 by using mini-Tn5 transposon mutagenesis.

Disruption of Colanic acid synthesis in *K. pneumoniae* enhances biofilm formation





## Methods

In this study, mini-Tn5 transposon insertion mutants were constructed and screened for their ability to form biofilms on abiotic surfaces by crystal violet staining. It was observed that disruption of *wcaJ* conferred high biofilm forming ability as compared to the parent strain. WcaJ is the initiating enzyme of colanic acid synthesis which loads the first sugar (glucose-1-P) on the lipid carrier undecaprenyl diphosphate. Its absence rendered the mutant defective in colanic acid, resulting in non-mucoid colonies. From the Calcofluor (binds cellulose) binding studies, it was evident that  $\Delta wcaJ$  mutant does not produce much exopolysaccharide, thereby facilitating cell-cell interactions leading to the formation of closely packed structures. In addition, complementation of *wcaJ* *in trans* fully restored the wild type phenotype that confirms the observed effect of *wcaJ* on biofilm formation of *K. pneumoniae*.

## Conclusions

Colanic acid expression in *K. pneumoniae* inhibits its cellular attachment to the substratum and biofilm formation, contradicting earlier studies that polysaccharides enhance biofilms in related species.

**FEMS-1420**

**Biofilms**

**SALT STRESS INDUCES BIOFILM FORMATION IN CLOSTRIDIUM LJUNGDAHLII.**

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<sup>1</sup>Labmet, Ghent University, Ghent, Belgium

<sup>2</sup>Microbiology Department, University of Massachusetts MA, Amherst, USA

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**Background**

*Clostridium ljungdahlii* is a homoacetogen capable of producing organic commodities from carbon dioxide using electrical energy provided by an electrode, i.e. microbial electrosynthesis. The efficiency of this process likely depends on the biofilm formation on the electrode. So far, however, only limited attachment to electrodes has been obtained with *C. ljungdahlii*, while biofilm formation by *C. ljungdahlii* has not yet been reported.

**Objectives**

The presented work investigated whether adapted growth conditions can induce *C. ljungdahlii* biofilms and characterized the obtained attachment.

**Methods**

*C. ljungdahlii* was grown in 6-well plates using a tryptone containing medium. Different stress conditions (salt, antibiotic, temperature) were tested and biofilm formation was quantified using a crystal violet assay. The addition of NaCl (200 mM) most significantly induced biofilm formation, i.e. after 3 days the attachment was two order of magnitudes higher than in the control. Confocal laser scanning microscopy showed viable,  $45 \pm 5$   $\mu\text{m}$  thick biofilms, while without the addition of salt only a single cell layer (5  $\mu\text{m}$ ) was observed. Furthermore, a  $15 \pm 5$   $\mu\text{m}$  thick biofilm was obtained on a vertically placed piece of graphite, simulating an electrode, by adding NaCl to the medium. RNA sequence data of *C. ljungdahlii* grown with and without the NaCl addition are currently being analyzed to explore the genes involved in biofilm formation.

**Conclusions**

This work is the first to demonstrate biofilms in *C. ljungdahlii* and suggests that salt-induced biofilms of *C. ljungdahlii* should be tested for their microbial electrosynthesis potential.





**FEMS-2205**

**Biofilms**

**CHARACTERIZATION OF XANA MUTANT OF THE XANTHOMONAS CITRI  
SUBSP. CITRI**

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**Background**

*Xanthomonas citri* subsp. *citri* is the causal agent of citrus canker disease. This bacteria is able to form biofilm in vitro and on leave surface. The ability to form biofilm is important to citrus canker development, because it implicates to survival of bacteria on leaf surface of host plants, when it is sprayed on the plant. Cells in biofilm are embedded in a matrix composed for extracellular polysaccharide (EPS) and other proteins. In *Xanthomonas campestris*, the gene *xanA* is required for the synthesis of the exopolysaccharide xanthan, but participation of *xanA* in biofilm formation process was not evaluated in *Xanthomonas citri*.

**Objectives**

To characterize the function of *xanA* (XAC3579) in relation to adhesion, biofilm formation, motility, and epiphytic behavior of *X. citri* on leaves.

**Methods**

We realized assays for analysis of biofilm formation and adhesion in abiotic and biotic surface, EPS quantification, sliding and swimming motility to compare the mutant XAC3579 tn5 and *Xanthomonas citri* wild type.

**Conclusions**

The reduction of xanthan produced reduced the adherence and also the swimming and sliding motility. The presence of the xanthan was required for mature biofilm development on sweet orange leaf surfaces. This reduction can decrease the *Xcc* pathogenicity.

**FEMS-0503**

**Biofilms**

**PROTEOMICS ANALYSIS IDENTIFIES PROTEINS ASSOCIATED WITH CURCUMIN-TREATED *HELICOBACTER PYLORI* BIOFILM**

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**Background**

*Helicobacter pylori* is an important human pathogen, which is implicated as etiologic agent of peptic ulcer, chronic gastritis and gastric cancer. Recently, *H. pylori* is shown to have ability to form biofilm both *in vitro* and *in vivo*. Biofilm formation enables the bacterium to survive in adverse environments, to persist in patients causing chronic infection and confers enhanced resistance to antibiotics. Curcumin (diferuloylmethane), a natural compound found in turmeric, has been demonstrated for broad chemotherapeutic properties. We have previously reported that curcumin inhibits biofilm formation of *H. pylori* but its impact at a molecular level is unclear.

**Objectives**

This study was established to identify proteins involved in anti-biofilm activity of curcumin in *H. pylori* biofilm using proteomics analysis.

**Methods**

Biofilm of *H. pylori* ATCC43504 was established by pellicle assay. Two-dimensional gel electrophoresis (2-DIGE) and matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) were performed in order to investigate the difference in protein profile expression between curcumin-treated and -untreated *H. pylori* biofilms.

**Conclusions**

There were total 28 expressed proteins identified here. Eighteen proteins were regulated in both curcumin-treated and -untreated *H. pylori* biofilms. Proteins with decreased expression following exposure to 1/4 MIC of curcumin were associated with carbohydrate and nitrogen metabolisms, chemotaxis and motility, and electron transport. However, several chaperon proteins were up-regulated in response to a presence of curcumin. These data reveal a probable mechanism of curcumin that inhibit biofilm formation of *H. pylori*. Further studies need to be investigated in order to develop curcumin as a potential alternative medicine for treatment of *H. pylori* infections.

FEMS-1966

Biofilms

**NOVEL METHODS FOR THE ASSESSMENT OF MARINE ANTI-BIOFILM TECHNOLOGIES.**

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**Background**

**Marine biofilms, mainly comprised of bacteria and diatoms, are now recognized to be a significant issue for a wide range of engineered structures such as ship hulls, energy systems, sensors, and oil/gas pipe lines. Biofilms constitute a major component of the overall marine growth on ship hulls and may lead to a 14 % increase in ship fuel costs and to an up to 18 % fuel consumption penalties. The scientific community is now placing significant effort towards the creation of environmentally benign surfaces with anti-biofilm capacity. However, there is significant gap in screening assays that test the bacterial/surface interface during initial attachment for anti-biofilm technologies while dynamic testing is often overlooked.**

**Objectives**

**In this work a range of bioassays have been developed to directly test bacterial attachment and biofilm growth on experimental anti-biofilm**

**surfaces, under both static and hydrodynamic conditions.**

#### **Methods**

**Following screening of several compounds for anti-biofilm efficacy, juglone (MIC at 5ppm) was chosen to act as model inhibitory agent, which was then successfully incorporated into a coating system and illustrated good anti-biofilm capacity. Novel microfluidic devices were developed for the assessment of bacterial attachments and biofilm formation in the presence and absence of juglone under hydrodynamic conditions. Juglone inhibited biofilm formation and following nucleic acid staining, toxicity against *Cobetia marina* was evident. This was not clear during the static assays.**

#### **Conclusions**

**The developed bioassays illustrated promising reproducibility and greater relevance for anti-biofilm technologies. New microfluidic approaches have been employed to reveal differences in results when comparing static vs. dynamic testing.**

## FEMS-2800

### Biofilms

#### **The impact of carbon source on biofilm formation, antigenicity and adhesion properties of the probiotic *Lactobacillus rhamnosus* GG**

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**Background.** Biofilm formation is the preferred growth mode and survival strategy of several bacteria for improving adaptation and viability under hostile conditions. For mucosal and health-promoting bacteria the biofilm formation is likely to play an important role in colonization and immunostimulation. We have previously shown that the probiotic paradigm *Lactobacillus rhamnosus* GG displays a high number of adhesive and immunostimulatory moonlighting proteins at the cell surface, and that surface-bound proteins are important mediators of biofilm formation of this strain.

**Objectives.** The aim of this study was to explore the effect of fourteen different fermentable carbohydrates on the biofilm formation of *L. rhamnosus* GG, and to uncover their impact on the surface protein/antigen composition and the adhesive properties of the strain during biofilm and planktonic growth.

**Methods.** Biofilm formation was assessed using the standardized polystyrene microtiter plate assay, and the viability and structure of selected biofilms were evaluated with LIVE/DEAD staining coupled with confocal microscopy analyses. Surface-associated proteins and antigens were identified and analyzed using in-house proteome and immunoproteome methods. Selected biofilm and planktonic cultures were further tested for their adhesion activities in vitro.

**Conclusions.** This study indicated that carbohydrate source plays a significant role in the protein-mediated biofilm formation and the antigenicity of the biofilm cells. We also show that moonlighting proteins are the major protein components of the biofilm matrix, and that certain carbohydrates affected the adhesion properties of biofilm cells.

## **FEMS-1307**

### **Biofilms**

#### **HIGH-THROUGHPUT SCREENING OF BIOFILM FORMATION BY AUTOMATED FLUORESCENCE MICROSCOPY**

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#### **Background**

Biofilms are communities of microorganisms which live in a self-produced matrix of extracellular polymeric substances. Their infections are hard to treat due to their resistance to immune defense and antibiotics. Therefore it is important to develop tools to investigate biofilms for therapy improvement or to find new antimicrobial substances.

#### **Objectives**

The aim of this work is to establish a method for the fully automated large-scale screening of biofilms which could be used for examining the ability of biofilm formation of bacteria under different culture conditions and most importantly for determining the effects of different antibiotics and antimicrobial substances.

#### **Methods**

We enhanced our previous published VideoScan technology, which is based on fully automated fluorescence microscopy to perform high-throughput screening of biofilms. VideoScan enables the analysis of multiplex assays such as microbead or cell-based assays [Rödiger et al. 2013; Frömmel et al. 2013]. We use a 96 well plate format for the formation of biofilms, which are stained with Live/Dead staining followed by a VideoScan analysis. This analysis represents a two-step evaluation realized by our software and image processing. In the first step overview pictures of biofilms are taken and in a second step, a fine-grained analysis of the biofilm, bacteria are counted in different z-stacks of the biofilm.

#### **Conclusions**

With our VideoScan technology it is possible to study biofilms in a fully automated large-scale screening. In a next step a large panel of commensal and pathogenic *E. coli* strains will be screened for biofilm formation under different growth conditions and presence of biofilm-associated genes.



**FEMS-1608**

**Biofilms**

## **CURCUMIN DERIVATIVES AS INHIBITORS OF BIOFILM FORMATION IN GRAM POSITIVE AND GRAM NEGATIVE BACTERIA**

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### **Background**

The development of alternative strategy to conventional antibiotics in the struggle against pathogens and antibiotic resistance is a topical issue. The interference with the adhesion, the first stage of pathogenic process, can modulate virulence mechanisms like colonization, invasion of host tissues and biofilm formation. The curcumin can inhibit the adhesion of both Gram positive and Gram negative pathogenic strains and different mechanisms can be involved (inhibition of sortase A, Quorum-sensing interference, binding to curli, etc.).

### **Objectives**

To obtain novel agents against pathogenic bacteria, targeting virulence mechanisms and biofilm formation (anti-virulence agents).

### **Methods**

In this study, we synthesized a group of molecules derivatives of curcumin, and the inhibition of biofilm formation was evaluated at a screening concentration of 7.5 µg/ml against reference staphylococcal strains *S.aureus* ATCC 29213, 25923, 6238, *S.epidermidis* RP62A, and Gram negative reference strains *E.coli* ATCC 10536 and *P.aeruginosa* ATCC 15442. The activity as sortase A inhibitors was also screened in a high throughput assay by using the standard Dabcyl-QALPETGEE-Edans fluorescence resonance energy transfer (FRET)-peptide substrate for measurement of enzyme activity.

### **Conclusions**

Some curcumin derived compounds resulted effective at lower concentration to prevent biofilm formation than the curcumin. The discovery of novel agents that target virulence mechanisms and biofilm formation offers new potential therapeutic strategies to treat chronic bacterial infections. They can act either alone or in combination with current antibiotics and could significantly impact on overcoming the problem of antibiotic resistance, which is recognized by WHO as one of the most important global challenges of our time.





**FEMS-2479**

**Biofilms**

## **UNRAVELING EROSION DYNAMICS: MICROBIAL STABILIZATION OF FINE SEDIMENTS**

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### **Background**

During the formation of aquatic biofilms microbes produce extracellular polymeric substances (EPSs) inter alia to attach to the sediment. Thus, sediment grains can be glued together and thereby are granted a significantly increased resistance versus erosive forces - a phenomenon called biostabilization.

### **Objectives**

This process constitutes an essential ecosystem function (e.g. detainment of contaminated fine sediments in rivers) but is currently barely addresses in lotic waters. In our current DFG project engineers and biologists work together to elucidate the fundamental principles of biostabilization.

### **Methods**

Straight flume mesocosm experiments were run at different seasons and under varied boundary conditions to investigate the effect of biotic and abiotic factors upon biofilm development and resulting biostabilization. Besides biofilms' stabilization capacity (adhesiveness and erosion resistance) biochemical and biological features (e.g. microbial biomass, EPS production and microbial community composition) were investigated.

### **Conclusions**

A general temporal increasing stabilizing effect could be detected - one of the first scientific proofs for this process in lotic waters. Furthermore, a strong effect of season and environment upon biofilm development and stabilizing capacity was detected, e.g. a significant maximum during spring but a minimum under minimal illumination. Besides the possible role of EPS protein content as proxy for biofilm stability the importance of successional processes were revealed: especially mutual adaption of algae and bacteria (e.g. expressed by the development of dynamics or functional organization within the bacterial community) is apparently a driving factor.

**FEMS-2981**

**Biofilms**

**CONFOCAL LASER SCANNING MICROSCOPY OF BIOFILMS OF EDNA-FORMING BACTERIA GROWN IN A CONTINUOUS FLOW BIOFILM REACTOR**

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**Background**

Recent studies show that extracellular DNA is a major component of the EPS (extracellular polymeric substances) in biofilms and it was found to have widespread importance in biofilm formation. However, detailed knowledge about how it promotes biofilm formation and how it influences biofilm morphology is just starting to emerge (1).

The  $\gamma$ -Proteobacterium F8 was isolated from the South Saskatchewan River in Canada. It was found to produce huge amounts of extracellular DNA forming a filamentous network when grown on agar plates containing FBM medium (2, 3).

**Objectives**

The aim of this study was to examine this phenomenon for the first time under continuous flow conditions in a biofilm reactor.

**Methods**

F8 was grown under controlled conditions in a continuous flow biofilm reactor and eDNA-production as well as biofilm morphology was monitored over time. Cells and EPS components were visualized by differential staining with various fluorescent dyes and confocal laser scanning microscopy (CLSM).

**Conclusions**

F8 cells attached to glass slides and showed biofilm formation from day 1 on. Filamentous eDNA could be detected by staining with Syto 9 and Propidium Iodide, but not with DAPI. Results so far indicate that eDNA is emerging from living cells.

**FEMS-1461**

**Biofilms**

**PSEUDOMONAS AERUGINOSA THAT COLONIZED BURN PATIENTS HAS HIGH CAPACITY TO FORM BIOFILM**

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**Background**

In burn patients, *Pseudomonas aeruginosa* can lead to a great variety of systemic infections with higher mortality rate. In addition, treatment of *P. aeruginosa* infections is a serious medical challenge because these bacteria are associated to high resistance to antibiotics and ability to form biofilms, becoming a serious problem in burn wound center (BWC).

**Objectives**

The aim of this study was to investigate the antibiotic resistance and biofilm formation from 35 *P. aeruginosa* collected from wound burn patients and on the surface of the bath tank where balneotherapy was performed.

**Methods**

The antibiotic resistance was carried out by disk diffusion method, following CLSI guidelines and the evaluation of biofilm formation was achieved through microtiter plate assay incubated for 24h. Genotypic characteristics of these strains were determined by Pulsed-field gel electrophoresis (PFGE).

**Conclusions**

It was observed that the clone A, the most prevalent and presents in wound patients and on the surface of the bath tank, had a high capacity to form biofilm. These strains also showed resistance to drugs as ceftazidime, aztreonam, gentamicin, ciprofloxacin and even meropenem. We believed that this clone which is more able to accumulate drug resistance mechanisms and to form biofilm has greater conditions to adapt and to persist in the BWC.

**FEMS-1575**  
**Biofilms**

**MICRO-DIVERSITY OF ANAMMOX BACTERIA IN NITRITATION-ANAMMOX BIOFILMS**

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**Background**

An emerging technology for nitrogen removal in wastewater is the Anammox process. However questions about the stability of the process remain. It is generally believed that a higher diversity can increase ecosystem stability. Coexistence of two anammox species in a Moving Bed Biofilm Reactor (MBBR) was observed in a previous study (Persson et al. 2014).

**Objectives**

We studied if coexistence of different anammox populations is common in nitrification-anammox MBBRs.

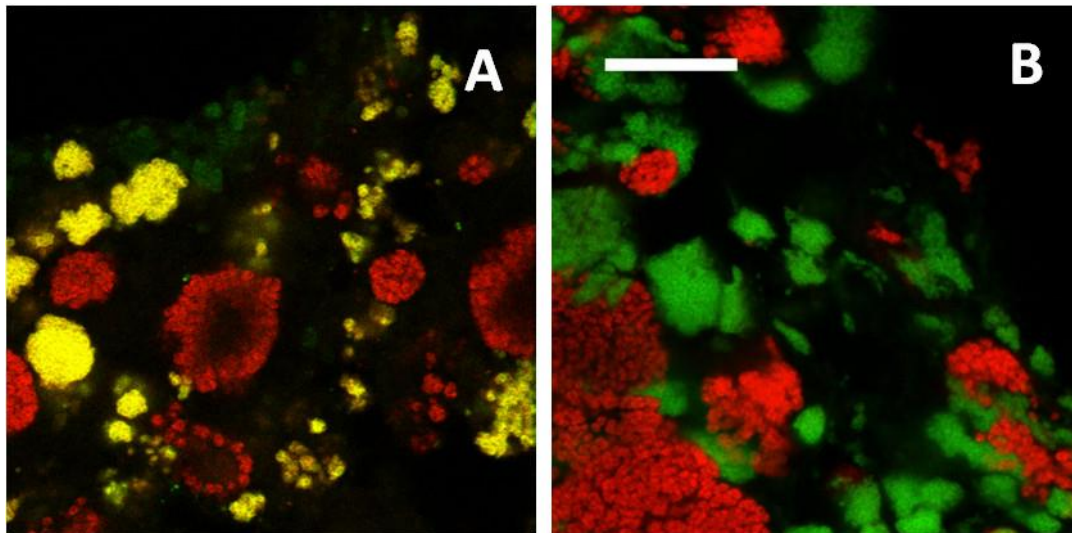


Figure 1: Different anammox populations (detected by different FISH anammox probes) coexisting in biofilms from two different MBBRs. Scale bar 20µm. A) Red: AMX820 probe, Yellow: Ban162 and AMX820. B) Red: Bfu613, Green: Ban162.

**Methods**

Anammox micro-diversity was assessed with automated ribosomal intergenic spacer analysis (ARISA) using a nested PCR approach, and fluorescence in situ hybridization (FISH). Four pilot MBBRs at two different pilot wastewater treatment plants in Sweden with different running conditions and one full-scale integrated fixed-film activated sludge reactor (IFAS) were studied.

**Conclusions**

Preliminary ARISA results suggest that nitrification-anammox MBBRs usually harbor more than one anammox population and FISH from two MBBRs confirmed this (Figure 1). Possible explanations include presence of biofilm microenvironments and temporal variations in environmental conditions such as influent composition. However, one MBBR operated at the challenging conditions of low nitrogen concentrations and at low temperature (13°C) was dominated by a single species.

**References**

Persson, F. et al. (2014). "Structure and composition of biofilm communities in a moving bed biofilm reactor for nitrification-anammox at low temperatures. *Biores. Technol.* 154: 267-273.

**FEMS-1719**

**Biofilms**

## **FUNCTIONALIZATION OF PDMS WITH CELLOBIOSE DEHYDROGENASE YIELDS ANTIBIOFILM SURFACE**

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### **Background**

Patients with medical indwelling devices (e.g. urinary catheters) are prone to acquiring infections caused by biofilm forming pathogens colonizing catheter surfaces. Catheter associated urinary tract infections (CAUTI) are among the most frequent ones. One of the polymers widely used for making urinary catheters is polydimethylsiloxane (PDMS) due to its favorable biocompatibility properties.

### **Objectives**

In order to decrease the risk of infection, an antimicrobial enzyme, namely cellobiose dehydrogenase (CDH) was successfully grafted onto PDMS surface.

### **Methods**

The system is based on the ability of CDH to use oxygen as electron acceptor and different oligosaccharides (e.g. cellobiose) as electron donors to produce H<sub>2</sub>O<sub>2</sub>. Several approaches of immobilizing CDH on PDMS surface were exploited including surface activation using oxygen plasma followed by covalent linkage of CDH. The success of the immobilization process was monitored by analyzing the change in the functional groups on the surfaces by FTIR measurements as well as measuring the ability of grafted CDH to produce H<sub>2</sub>O<sub>2</sub>. Antimicrobial activity of the immobilized enzyme was assessed against *S. aureus* by quantifying the amount of viable cells and total biomass formed on the enzyme treated sheets compared to the control.

### **Conclusions**

CDH was successfully immobilized on the surface of PDMS as evidenced by H<sub>2</sub>O<sub>2</sub> production in the presence of cellobiose. The modified surface showed up to 70% reduction of microbial growth as compared to the untreated control. The CDH modified PDMS catheters could help to prevent current problems of microbial colonization and multidrug resistant bacteria associated with catheters.





**FEMS-2513**

**Environmental microbiology - 2**

**ENHANCED FLOC FORMING MUTANTS OF RHODOVULUM SULFIDOPHILUM  
OBTAINED BY UV MUTAGENESIS**

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**Background**

A marine photosynthetic bacterium, *Rhodovulum sulfidophilum*, produces nucleic acids extracellularly<sup>1</sup>). Interestingly, the nucleic acids thus produced outside cells are utilized as a sticky glue to make bacterial self-aggregates called “floc” in nutritionally poor medium conditions such as on the sea bottom where the bacterium was originally isolated. However, what gene regulates its flocculation or why this bacterium makes a floc under nutritionally stringent conditions is still unclear.

**Objectives**

Our objectives are to establish UV mutagenesis in *Rhodovulum sulfidophilum* and to obtain UV mutagenic clones showing aberrant flocculation behavior even in rich medium conditions where the bacterium usually doesn't make an obvious floc.

**Methods**

*Rhodovulum sulfidophilum* DSM 1374<sup>T</sup> was exposed to UVC (254 nm) irradiation and UV mutagenesis was induced. After UVC irradiation, rifampicin-resistant mutants were collected and these flocculation behaviors were examined.

**Conclusions**

UV mutagenesis in *Rhodovulum sulfidophilum* DSM 1374<sup>T</sup> was established and 107 rifampicin-resistant mutants were obtained. Two out of the obtained clones showed strong flocculating behavior even in nutritionally rich medium conditions and these flocculation ratios were approximately 40% and 60%, respectively, whereas that of wild type was ca. 20%.

**FEMS-2922**

**Biofilms**

## **MUTUAL INFLUENCE OF *S. EPIDERMIDIS* AND *S. AUREUS* ON GENE EXPRESSION IN DUAL SPECIES BIOFILMS**

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### **Background**

*S. aureus* and *S. epidermidis* are two important nosocomial pathogens which can interact in biofilms (e.g. *S. epidermidis* inhibits *S. aureus* biofilm formation by producing proteases).

### **Objectives**

To study the mutual effect of *S. epidermidis* and *S. aureus* on gene expression in dual species biofilms.

### **Methods**

RNA-Seq was performed on single and dual species biofilms and the results were confirmed by the appropriate phenotypic tests.

### **Conclusions**

In dual species biofilms, *S. epidermidis* genes encoding resistance to erythromycin, oxacillin and tobramycin were upregulated and when biofilms were treated with these antibiotics, we observed that more *S. epidermidis* cells survived oxacillin treatment in dual species biofilms than in single species biofilms.

Urease genes of *S. aureus* were downregulated in dual species biofilms and an urease assay confirmed that there was more urease activity in *S. aureus* single species biofilms compared to dual species biofilms. Literature data described a link between urease activity and pH and we also observed that the pH of *S. aureus* single species biofilms was lower than the pH of dual species and *S. epidermidis* single species biofilms. Metabolic activity results in acid production and RNA-Seq data showed there was less expression, in dual species biofilms, of *S. aureus* genes encoding proteins involved in metabolic processes. Viability assays showed that there were more metabolically active cells in *S. aureus* biofilms compared to dual species and *S. epidermidis* single species biofilms.

Altogether, our results show that *S. epidermidis* has an inhibitory effect on the metabolic activity of *S. aureus* in dual species biofilms.



**FEMS-0837**

**Biofilms**

**ANTI-BIOFILM EFFECT OF RED RASPBERRY FRUIT JUICE ON  
PSEUDOMONAS AERUGINOSA BIOFILM**

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**Background**

Bacterial cells in biofilm are more resistant to wide range of disinfectants than free living cells, and finding an appropriate agent against them is demanding task.

Environment is certainly the source of many products which have anti-biofilm effects, but they have not been proven, yet.

**Objectives**

To investigate the anti-biofilm effect of 100% pure red raspberry fruit juice on *Pseudomonas aeruginosa* biofilm formation.

**Methods**

Minimal inhibitory concentration of red raspberry fruit juice was evaluated by dilution method, and its anti-biofilm effect was tested in microtiter plate using crystal violet staining according to modified technique by Stepanovic.

**Conclusions**

Out of 30 tested strong-adherent *Pseudomonas aeruginosa* isolates, after treating with red raspberry fruit juice, 19 (73.3%) isolates were poorly adherent, while 8 (26.7%) had no adherence ability.

This study confirmed the great potential of red raspberry fruit juice as anti-biofilm agent for preventing microbial colonization, but it is necessary to discover the exact biochemically active ingredient against biofilm formation.

**FEMS-2613**

**Biofilms**

**ADVANCEMENTS TOWARDS A SYSTEM-LEVEL UNDERSTANDING OF  
SUBAERIAL BIOFILMS INHABITING OUTDOOR STONE MATERIALS**

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**Background**

Many of the world's most precious artworks are made of stone. Their irreversible deterioration due to biological attack is a worldwide concern. Microorganisms colonize outdoor lithic surfaces and develop into biofilms at the interface solid/air (subaerial biofilms, SABs), which, in turn might cause aesthetic, chemical and physical decay. Although it has been estimated that at least 99% of the world's microbial biomass exists in biofilms, the role and behavior of microorganisms within the biofilm matrix and their complex interactions with the external environment is still unknown.

**Objectives**

This work provides a pioneering and multidisciplinary research to investigate the behavior of microorganisms within the biofilm matrix for sorting out time-spatial relationships and to elucidate microorganism-EPS, inter-organism, biofilm-atmosphere and biofilm-stone interactions.

**Methods**

This work spans sophisticated molecular, chemical, physical and data modeling techniques and it is approached from two complementary angles:

1- Lab-scale study to delineate specific transcriptional responses of mono- and multi-species biofilms as well as the biofilm-stone interactions under controlled environmental conditions.

2- Real heritage case studies to investigate the shifts in the microbial community structure and function under different environmental conditions. Through comparing phylogenetic and functional diversity under different environmental scenarios, we

provide evidence that any intuition gained from the lab-scale experiments is relevant to true environmental biofilms.

## **Conclusions**

The findings obtained so far will contribute to better understand the complexity of all the interactions encountered within SAB communities, and how these interactions may influence the biofilm outcome and the biodeterioration of the stone materials under different environmental conditions.

**FEMS-1503**

**Biofilms**

## **BIOLOGICAL FUNCTIONS AND CELLULAR LOCATION OF COLLAGEN-LIKE PROTEINS IN BACILLUS AMYLOLIQUEFACIENS**

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### **Background**

Bacterial collagen-like proteins (CLPs) have been identified in a broad range of pathogen bacteria and it is important to biofilm formation and bacterial adhesion to host cells. Some bacterial CLP-encoding genes (clps) have also been found in non-human pathogenic strains such as *B. cereus* and *B. amyloliquefaciens*, which are types of plant-growth promoting rhizobacteria (PGPR).

### **Objectives**

To elucidate the role of CLPs playing in the interaction between PGPR with plant host, the biological functions and cellular location have to explore.

### **Methods**

CLP-encoding genes ClpA, ClpB, ClpC, and ClpD in strain FZB42, were inactivated separately by Site-Directed Mutagenesis. Scanning electron microscopy (SEM) and hydrophobicity value detection were used to assess the morphologic bacterial cell shape and cell surface architecture. Immune gold labeling was used to indicate the location of CLPs on the cells. The bacterial flagella morphology observed by transmission electron microscopy (TEM).

### **Conclusions**

Comparing with wild type strain, clps mutant strains showed differently on phynotypes of bacterial colony shape, cell autoaggregation, biofilm formation, as well as adhesion to surface of abiotic materials or the roots of *Arabidopsis thaliana*. Immune gold labeling shown CLPs located in the outer layer of the bacterial cell, including the cell wall, outer membrane, flagella, or other associated structures. In addition, the bacterial flagella appeared fewer, scattered and bent for clps mutant strains. Above

results indicate that the bacterial out layer located CLPs are closely involved in cell shape development, biofilm formation, and bacteria-plant interaction.



**FEMS-3135**

**Biofilms**

**SYSTEMS MICROBIOLOGY OF ALGINATE-LIKE EXOPOLYSACCHARIDES BIOSYNTHESIS IN GRANULAR SLUDGE BIOFILMS: A PILOT AND FULL-SCALE STUDY WITH DOMESTIC WASTEWATER**

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**Background**

Environmental biotechnology systems using aerobic granular sludge (AGS) are becoming new standards for intensified wastewater treatment. AGS originates from self-aggregation of activated sludge microorganisms into mobile biofilm structures under operational selection pressures. Extracellular polymeric substances (EPS) such as alginate-like exopolysaccharides (ALE) play an important structural role by supporting microbial encapsulation in a gel matrix.

**Objectives**

The complex microbiome of AGS was studied using a systems biology approach. Investigations targeted bacterial phylotypes carrying genetic and metabolic potential for ALE biosynthesis. Such correlation analysis will enable to elucidate molecular mechanisms underlying granular biofilm formation.

**Methods**

A 98-L pilot sequencing-batch reactor was operated for granulation and nitrogen removal from domestic wastewater. Evolution of particle size distribution and EPS polysaccharide and protein fractions were determined by laser diffraction, phenol-sulfuric acid, and folin-phenol methods, respectively. AGS from full-scale system was subjected to physicochemical characterization of ALE. Bacterial populations and genetic signatures related to ALE biosynthesis are currently prospected from pilot and full scales using phylogenetic and metagenomic profiling.

**Conclusions**

Over 3 months of operation granulation displayed gradual increase in the fraction and mean diameter of granular biofilms from 5 to 60% and 50 to 300  $\mu\text{m}$ , respectively. The polysaccharide-to-protein ratio increased concomitantly from 0.4 (m/m) in activated sludge flocs to 0.8 and 1.0 in nitrifying and denitrifying granules, respectively. Lyophilized granules from full-scale plant contained 15% of ALE, i.e. twice higher than typically measured in flocs. Correlating these physicochemical gradients to phylogenetic structure and genomic signatures will allow for fundamental understanding of the granulation phenomenon and its temporal triggering.

**FEMS-1214**

**Biofilms**

**INFLUENCE OF COPPER CHLORIDE AND COPPER SULFATE ON BIOFILM FORMATION OF TWO STAPHYLOCOCCUS AUREUS STRAINS**

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**Background**

Biofilms pose a way to overcome toxic influences like antibiotics and metal ions. As the number of multiresistant bacteria increases (e.g. *Staphylococcus aureus*) steadily, metals (copper, silver) celebrate their renaissance/revival for many purposes in health care facilities. Not much is known about the tolerance against these heavy metals.

**Objectives**

The known positive effect of the heavy metal tolerance of formed biofilms implicates that there could be a regulatory effect of copper ions on the formation and maturation of biofilms. Previous studies on this matter present inconclusive data, pointing in both directions.

**Methods**

The influence of different CuCl<sub>2</sub> and CuSO<sub>4</sub> concentrations (0.5 – 5 mM) on the formation of biofilms of two *S. aureus* strains as well as on already formed biofilms was tested in a multi well plate assay. Protein and polysaccharide concentrations were quantified and biofilm growth was measured after 48h and 96h.

**Conclusions**

If grown in copper containing solutions the both strains used showed a different behavior. One strain was inhibited at higher copper concentrations (> 2.5 mM), the other strain presented an increased EPS production at the same level. Both, CuCl<sub>2</sub> and CuSO<sub>4</sub>, did not show an inhibitory effect on already formed biofilms of both strains. Interestingly lower concentrations (<3 mM) revealed a biofilm promoting effect. Further studies with more strains are needed to be certain which influence copper has on biofilms and their formation as apparently the strains show different behavior.

**FEMS-2992**

**Biofilms**

## **DEVELOPMENT OF BIOFILM MATRIX AND CELL-CELL COHESION IN RHIZOBIUM LEGUMINOSARUM**

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### **Background**

Biofilm formation is important for rhizobia to colonize several environmental niches. Within the microcolonies developed by *Rhizobium leguminosarum*, rhizobial cells interact tightly through lateral and polar connections forming compact aggregates. These microcolonies are surrounded by a biofilm matrix, whose main component is the acidic exopolysaccharide (EPS). In addition, the PrsDE secretion system has been implicated in biofilm formation. This system is responsible for the secretion of Rap proteins that share one or two Ra/CHDL (cadherin-like-) domains.

### **Objectives**

We aimed to explore the role of Rap(s) and other surface factors in biofilm development.

### **Methods**

RapA was analyzed by biophysical and biochemical approaches. Functional studies were assessed by mutagenesis and gene overexpression.

### **Conclusions**

We showed that RapA, which consists of two Ra/CHDL domains, is a unipolar calcium dependent lectin that specifically binds the EPS. RapA overexpression enhanced biofilm formation and expanded the distance between cells. In line with these observations, we showed that high levels of RapA secretion increases capsular polysaccharide (CPS) formation, which is structurally and genetically related with the EPS. We propose that RapA and the other Rap proteins, which are predicted to harbor one or two EPS/CPS-binding domains, play some role remodeling the biofilm matrix structure. Exploring the role of another important surface polysaccharide, the lipopolysaccharide (LPS), we found that the O-chain core region of the LPS is crucial for cell-cell cohesion. Mutants defective in the O-chain core moiety developed

biofilms with an altered three-dimensional structure. Further studies are required to give insight into the interplay between the biofilm matrix components and Rap(s).

**FEMS-2762**

**Biofilms**

## **BIOFILM ACCUMULATION AT DIFFERENT WATER TREATMENT STAGES**

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### **Background**

Biofilms are microbial communities and highly relevant for water quality. In principle biofilms may affect human health in drinking water reservoirs and distribution systems hindering the efficient operation of these systems. Also they may also pose a health risk due the presence of pathogenic bacteria such as *Legionella pneumophila* and *Escherichia coli*.

### **Objectives**

The aim of this study was to describe biofilms accumulating within controlled pilot plant after different treatment steps. The use of various methodological approaches should describe biofilms and the associated water phases in order to assess potential factors associated with human health risks.

### **Methods**

In total 6 biofilm samples were collected from the PE-X pipes of the pilot drinking water treatment plant.

The plant is designed for the drinking water treatment of a small rural community located in Upper-Austria (193 inhabitants in 2014; average daily water consumption: 20 m<sup>3</sup>). The treatment has 4 successive steps: 1) oxygen enrichment (using pressurized air) 2) biological nitrification, 3) rapid sand filtration and 4) UV-disinfection.

Biofilms were characterized by chemical (TOC, DOC, heavy metals), microbiological (HPC, flow cytometry) and molecular biological (16S rRNA gene sequencing) and microscopic methodologies.

### **Conclusions**

The multi-parametric approach applied in this study has shown valuable results with good correlations among chemical and microbiological characterization. The activity of biofilms (viability) could be described by flow cytometry and fluorescent microscopy. Phylogenetic analysis of sequenced culturable fractions revealed differing diversities in the microbial communities.

**FEMS-0996**

**Biofilms**

**Novel strategies for prokaryotes to rapidly transition from planktonic cells to life in a biofilm**

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Biofilms are diverse microbial communities, bound by a matrix of extracellular polymeric substances, that allow cells to survive a variety of stressful, potentially lethal, conditions. To successfully avoid exposure to such toxic conditions, cells must transition rapidly from planktonic to sessile state, requiring quick acting regulatory mechanisms that can both inhibit motility and promote adhesion. We previously showed that the evolutionarily conserved type IV pili (TFP) are crucial for surface adhesion and microcolony formation in the model archaeon *Haloferax volcanii*. We also demonstrated that six conserved *H. volcanii* pilus subunits, the pilins PilA1-6, are involved in the regulation of flagella-dependent motility. Additionally, a subset of these pilins promotes microcolony formation, while at least two of the remaining pilins inhibit it. Recent studies suggest that pilin glycosylation catalyzed by the glycosyltransferase, AglB, plays an important role in the regulation of microcolony formation. Consistent with the pilins required for microcolony formation (PilA5 and PilA6) not being glycosylated, and the remaining pilins requiring glycosylation to function properly, we have shown that a  $\Delta$ aglB strain forms microcolonies. Stress adversely affects AglB-dependent glycosylation, which is also required for flagella-dependent motility, hence inhibiting motility and promoting microcolony formation. Consistent with this we found that under low salt conditions *H. volcanii* is not motile and readily forms microcolonies. This differential post-translational modification that allows *H. volcanii* cells to respond quickly to changes in the environment that result in conditions favorable to sessile cells and biofilm formation rather than motile planktonic cells may be broadly conserved across prokaryotes.

**FEMS-2807**

**Biotechnology and industrial microbiology**

**The potential of halophilic microorganisms to convert waste glycerol to valuable compounds: glycerol carbonate (GlyC) and glycidol (GlyD)**

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Background: Waste glycerol is the most important by-product in the production of biodiesel from biomass. As a direct consequence, waste glycerol (unpurified) was produced in quantities which soon will exceed current market demands leading to serious environmental problem by its storage.

Objectives: This work enlarges the application area for waste glycerol, proposing its usage as raw material in the production of glycerol derivatives (e.g. GlyC, GlyD and polyglycidol). Thus, alternative solutions for the storage problem of waste glycerol that can generate negative effects on the environment are developed. GlyC, GlyD and polyglycidol represent products of interest for fine chemical industry. Thus, GlyC is used as solvent for cosmetics and in medicine, while GlyD and polyglycerols appear as synthesis intermediates of plastics and resins in the polymer industry, but also in the pharmaceutical and cosmetic industries.

Methods: The products of interest were revealed in culture media using GC-MS and GC-FID techniques. Also, the polyglycidol products were evaluated (Mw, Mn and PI) based on GPC technique.

Conclusions: Preliminary results revealed ability of various moderately halophilic bacteria isolated from saline and hypersaline environments from Romania to convert waste glycerol to GlyC and GlyD. Lipase enzyme is responsible for GlyC production, while lipase and decarboxilase mixture convert the glycerol to GlyD. Polymerization of GlyD is performed under basic conditions.



**FEMS-2821**

**Biotechnology and industrial microbiology**

**Production of antimicrobial and anticancer substances by *Bacillus pumilus* SG-32 and *Bacillus firmus* P1-1 isolated from oil reservoirs in Brazil.**

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**BACKGROUND:** Thirty strains isolated from Brazilian oil reservoirs were tested against each other for the production of antimicrobial substances. Two strains of *Bacillus*, *B. pumilus* SG-32 and *B. firmus* P1-1 were selected due to their ability to inhibit more than 60% of the strains tested. These strains were also investigated for cytotoxic activity against human cancer cell lineages.

**METHODS:** The screening of antimicrobial substances was performed by the agar diffusion method described for Rosado and Seldin (1993). Thirty strains previously isolated from oil reservoirs in Campos Basin (RJ, Brazil) were tested against each other for antimicrobial production. Two selected strains were grown in Marine Broth for 72 hours at 40°C to increase cell biomass. Anticancer screening was performed against nine different types of human cancer cell lineages.

**RESULTS:** Among the thirty strains tested, three of them (two strains of *Bacillus pumilus*, SG-30 and SG-32, and one strain of *Bacillus firmus*, P1-1) were able to inhibit more than 60% of the bacterial strains under study. Strains P1-1 and SG-32 were chosen for subsequent cytotoxic activity assay against human cancer cells. Strain P1-1 presented better effect than the positive control doxorubicin against colon and breast cell cancer lineages. Strain SG-32 was not as efficient as the positive control; nevertheless five types of cancer cell lineage were inhibited.

**CONCLUSIONS:** Three different *Bacillus* strains from Brazilian oil reservoirs were able to produce antimicrobial substances, two of them with significant activity against cancer cell lineages, showing potential for further clinical trials.

**FEMS-0500**

**Biotechnology and industrial microbiology**

**SAFETY EVALUATION OF SOME COMMERCIAL SKIN COSMETICS USING A GENETICALLY MODIFIED ACINETOBACTER BIOLUMINESCENT BIOREPORTER DF4/PUTK2**

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**Background**

Human skin is suffering from continued use of cosmetics, which may leads to ecosystem disruptions of useful skin microbiota. Therefore, a need to ensure its safety-in-use is important. Despite, microbiological assays employed to determine the lowest cosmetic concentration gave the lowest microbial contamination level during usage; toxicity assay must be performed first.

**Objectives**

In the present work, the Bioreporter DF4/PUTK2 was employed to assess the toxicity of eight local commercial cosmetic products. DF4/PUTK2 is a genetically modified strain belongs to the genus *Acinetobacter* that widely spread in nature and skin microbiota. Previously, DF4/PUTk2 was successfully employed to assess toxicity of phenolics and heavy metals.

**Methods**

Present results indicated that among the eight tested cosmetics, the Bioreporter was directly sensitive in order of decreasing sensitivity to aftershave balm, skin-refining toner, moisturizing cream and purifying cream with bioluminescence inhibition percent (BI%) equal to 64%, 58%, 50% and 30% respectively, Followed by eye makeup remover and sun cream with (BI%) equal to about 15% but after exposure time 300 and 700 min respectively. However, no decrease for bioluminescence with eye color powder and color trend – fresh foundation was observed. Also, the effect of exposure time on bioluminescence was tested. It was noticed that the cosmetics that expected to remain for a long time on skin seems most toxics.

**Conclusions**

In conclusion, the Bioreporter DF4/PUTK2 can be used to assay the toxicity of cosmetics and it is candidate to be a prescreening and quality control tool to pre-select the most toxic cosmetic for further chemical analysis.

**FEMS-0528**

**Biotechnology and industrial microbiology**

**INCREASING SOLUBLE EXPRESSION OF ALPHA-LUFFIN RIBOSOME  
INACTIVATING PROTEIN BY ENBASE FED-BATCH CULTURE**

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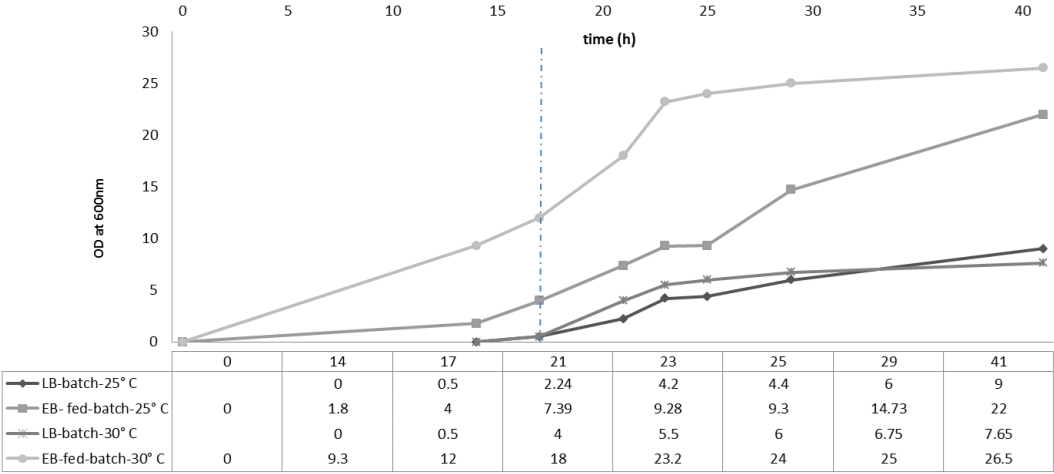
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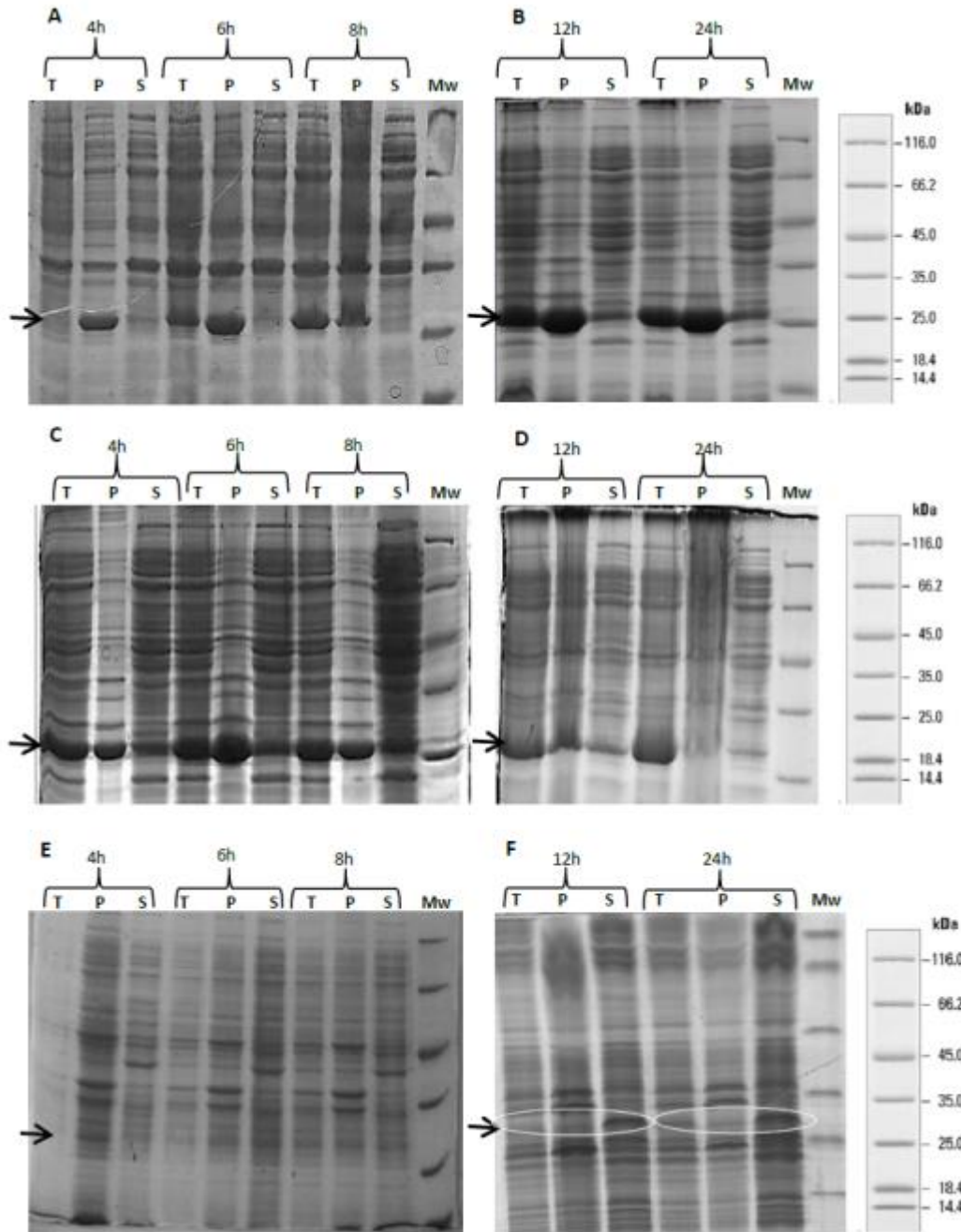
**Background**

Considering the undeniable advantages of natural toxins from RIP family to produce anticancer immune-conjugates, well optimized recombinant expression of this category of proteins is of importance regarding industrial aspects. One of the proposed cellular mechanisms involved in EnBase fed-batch mode of cultivation is the change in protein expression via ribosomal manipulation. However, this system has not been applied to cytotoxic proteins.

**Objectives**

For the first time here, the expression profile of  $\alpha$ -luffin, a ribosome inactivation protein (RIP) with an innate toxicity, was optimized in EnBase system. In fed-batch fermentation mode, the early incubation time was preferable at 30° C culture temperature whereas at 25° C the extended protein synthesis period (12 and 24h post induction) resulted in higher amount of soluble recombinant protein.





## Methods

Mature  $\alpha$ -luffin encoding cDNA was synthesized and subcloned into pET28a under the control of T7 promoter fused to the 6-HIS tag. The *E. coli* expression procedure was compared in traditional batch and newly developed fed batch; EnBase® Flo system at 25° C and 30°C incubation temperatures.

## Conclusions

As the first study investigating the efficacy of EnBase fed-batch mode for the production of a toxic protein ( $\alpha$ -luffin) it is concluded that in spite of the efficacy of this system in producing higher soluble protein ratios compared to batch cultivation growth rate, incubation temperature and time need to be optimized so as to cover innate cytotoxicity. The optimized condition proposed here is promising in terms of large scale soluble production of  $\alpha$ -luffin without the need for refolding.

**FEMS-0563**

**Biotechnology and industrial microbiology**

## **ANALYSIS OF TRANSCUTANEOUS DELIVERY OF TAT-BONT/A(1-448) FUSION PROTEIN AS THERAPEUTIC AGENT**

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### **Background**

Botulinum neurotoxin type A (BoNT/A) composed of three domain. One catalytic domain and one binding domain which are linked together by a translocation domain. In recent years, BoNT/A was used as a therapeutic agent for treatment some abnormal muscle contractions such as strabismus, blepharospasm, spasmodic or face wrinkles caused by ageing process. The injection of this drug causes some undesirable side effects such as irritation, pain, bleeding at the point of injection. Cell penetrating cationic peptides (CPPs) are three to 30 peptide residues which freely pass through cell membranes. Tat peptide is a CPP which is a favorite way to transduction of biological macromolecules (peptides, proteins or nucleotides) into cells.

### **Objectives**

In this study, Tat<sub>(47-57)</sub> peptide directly fused to catalytic domain of BoNT/A. The penetration of this recombinant protein was analyzed *in vitro*.

### **Methods**

A genetic construct containing Tat gene sequence, residues 47-57, was fused with that of BoNT/A (residues 1-448) and cloned into a bacterial host for further expression. Obtained fusion protein, was purified and transferred into a 6 well plate seeded by HeLa cell line and was incubated at 37°C for 1 to 2 h. Transduction of protein was further analyzed by western blot.

### **Conclusions**

Results showed that Tat<sub>(47-57)</sub> peptide can carry the BoNT/A<sub>(1-448)</sub> into HeLa cell line in comparison to BoNT/A<sub>(1-448)</sub> as control. Cell transduction was time dependent (protein band in western blot after 2 h was thicker than 1 h). CPPs are promising tools for

transferring different type of drugs directly into intact skin for therapeutic purposes through a non-invasive way.



**FEMS-0568**

**Biotechnology and industrial microbiology**

## **CAN *S. CEREVISIAE* PRODUCE BIO-BASED POLYMERS FROM PENTOSE SUGARS?**

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### **Background**

A sustainable future necessitates an urgent transition from petroleum-based industries towards the production of bio-based bulk chemicals from renewable feedstock [1]. The bio-based polymers, such as polyhydroxyalkanoates (PHAs), can be produced from monomeric sugars. Poly-3-D-hydroxybutyrate (PHB) is one of the most studied PHAs and is a promising biopolymer produced naturally by several bacterial species [2]. In parallel, xylose is the main pentose sugar present in lignocellulosic biomass (LCB), which is considered the cheapest carbon source on earth [3]. Thus, LCB is a potential substrate for the bio-based production of bulk chemicals such as PHB.

### **Objectives**

In this work, the capacity of recombinant *Saccharomyces cerevisiae* to produce PHB using pentose sugars (xylose) as the main carbon source was evaluated.

### **Methods**

Engineered *S. cerevisiae* strains, capable of pentose utilization were transformed with the PHB pathway genes from the natural and well-characterized PHB-producer *Cupriavidus necator*. The two host strains carried genes for xylose utilization from the yeast *Scheffersomyces stipitis*, but two variants of the xylose reductase (XR) with different NADH/NADPH preference ratio were tested [4]. The resulting strains were evaluated for their PHB-production capacity.

### **Conclusions**

The production of PHB from xylose-rich medium was achieved using *S. cerevisiae*. The highest PHB yield (g/g xylose) was achieved with the strains carrying the mutated *S. stipitis* XR with a higher NADH/NADPH preference, highlighting the importance of redox balancing for PHB production.

[1]J.G. Lambert et al., Energy Policy 64:153-167, 2014.

[2]K.Kocharin et al., AMB Express 2:1-11, 2012.

- [3] R.G. Sanchez et al., *Biotechnol. Biofuels* 3:3-13, 2010.
- [4] D. Runquist et al., *Appl. Environ. Microbiol.* 76:7796-7802, 2010.

**FEMS-0597**

**Biotechnology and industrial microbiology**

## **USE OF FUNGAL ROTATING DISK REACTOR (RDR) FOR BIODEGRADATION OF TEXTILE ANTHRAQUINONE DYES**

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### **Background**

Biodegradation potential of fungi for removal of dyes from textile wastewater has been established and applied in aerated reactors.

### **Objectives**

The aim was to compare dye-degradation efficiency of a newly isolated *Trametes suaveolens* strain with different fungal species in RDR. Reactive RB19 (C.I.61200) and disperse DB3 (C.I.61505) dyes were used.

### **Methods**

2-L RDR (6 disks, rotation speed 2 rpm, air flow 50L.h<sup>-1</sup>) was used for dye decolorization (50mg.L<sup>-1</sup>) in malt extract-glucose medium at 22°C. Decolorization was measured spectrophotometrically and the end-products by GC/MS. Bacterial luminiscence test, plant growth tests and Ames test were used to measure acute toxicity and genotoxicity.

### **Conclusions**

Using batch mode, RB19 decolorization rates were: *Irpex lacteus* > *T. suaveolens* > *Pleurotus ostreatus*. When continuous mode was used, the dye degradation capacity of *T. suaveolens* was 2-fold compared to the batch mode. Majority of the degradation products were aromatic alcohols. No genotoxicity was detected after degradation. The acute toxicity measured with *Vibrio fischeri*, *Sinapis alba* and *Lemna minor* decreased 1.2-, 2.7- and 1.6-fold, respectively. The results demonstrated the efficiency of fungal RDR for removal of textile anthraquinone dyes.

Supporting projects: IAAX00200901, AV0Z50200510, IET CZ.1.05/2.1.00/03.0100, OPVK CZ 1.07/2.3.00/30.0019.



**FEMS-0607**

**Biotechnology and industrial microbiology**

## **SULFATE-REDUCING BACTERIA AS WHOLE-CELL BIOCATALYSTS FOR HYDROGEN PRODUCTION**

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### **Background**

Sulfate-reducing bacteria (SRB) are a major group of environmental bacteria with important biotechnological applications in waste water treatment and bioremediation. These organisms have an extremely high hydrogenase activity and in natural habitats where sulfate is limited they produce hydrogen fermentatively and grow syntrophically with other organisms. However, their potential as H<sub>2</sub>-producers has been poorly investigated.

### **Objectives**

Given the high number of hydrogenases present in SRB genomes [1] we explored the potential of the model strain *Desulfovibrio vulgaris* Hildenborough for H<sub>2</sub> production from three substrates (lactate, ethanol, formate).

### **Methods**

Among the substrates tested the highest H<sub>2</sub> production was observed from formate [2]. Formate has emerged recently as an environmental friendly storage of H<sub>2</sub> that can be easily transported and has relatively low toxicity. Therefore a lab-scale H<sub>2</sub> production process with gas sparging was designed to evaluate the potential of SRB as biocatalysts for formate-driven H<sub>2</sub> production [3].

### **Conclusions**

By optimizing the operation conditions of bioreactor such as temperature, gas flow rate and cell load, a high H<sub>2</sub> production rate was obtained. In addition, a high specific hydrogen production rate and 100% efficiency of substrate conversion were achieved, demonstrating the high potential of SRB for H<sub>2</sub> production from formate [3]. Our studies reveal that SRB can be used as an efficient biocatalyst for the conversion of formate to hydrogen.

[1] Pereira IAC et al (2011) Front Microbiol 2, 69

[2] Martins M et al (2013) Int J Hydrogen Energy 38, 12294

[3] Martins M et al (2014) submitted



**FEMS-0617**

**Biotechnology and industrial microbiology**

## **INVESTIGATING MICROALGAE AS A BIOTECH PLATFORM FOR HIGH-VALUE OILS**

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### **Background**

Natural oils are found in a diverse range of products such as soaps, paints, cosmetics, antibacterial treatments and nutritional supplements (fish oils).

### **Objectives**

As part of a consortium with the University of Cambridge, the University of Aberdeen and Rothamsted Research we aim to establish the microalga *Nannochloropsis gaditana* as a platform for the production of these commercially important oils. The advantages of microalgae as a production platform include low media costs, lack of toxins and fast growth rates; *N. gaditana* was chosen due to its naturally high lipid content.

### **Methods**

The production of bespoke lipids will require a combination of metabolic modelling and nuclear genetic engineering to introduce or knock out specific elongase and desaturase genes. To test the genetic tractability of this species, two constructs were prepared that contained *ble-gfp* or *ble-HA* fusion genes; the *ble* marker encodes resistance to zeocin. *N. gaditana* was transformed by electroporation and colonies were selected on media containing zeocin. All of the colonies tested carried the expected transgene and accumulated Ble-GFP or Ble-HA fusion protein, as detected by western blotting.

### **Conclusions**

The *N. gaditana* nuclear genome is amenable to genetic transformation by random integration, paving the way for our metabolic engineering studies over the next three years.

**FEMS-0634**

**Biotechnology and industrial microbiology**

## **MALIC ACID PRODUCTION FROM RENEWABLE SOURCES BY ASPERGILLUS ORYZAE**

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### **Background**

L-malic acid is a C4 dicarboxylic organic acid and considered as a promising chemical building block. It can be applied as food preservative and acidulant, in rust removal because of its chelator properties and as polymerization starter unit due to its bifunctionality. Up to now it is produced chemically from crude oil via maleic anhydride. The mould *Aspergillus oryzae* produces malic acid in large quantities from glucose and other carbon sources. The microbial production of organic acids from renewable sources has the potential to be a sustainable alternative to petroleum and to reduce greenhouse gases as CO<sub>2</sub> fixation is involved in microbial biosynthesis.

### **Objectives**

The potential of malic acid production from renewable resources is evaluated. Therefore, different carbon sources based on lignocellulosic biomass, e.g. fractions of pyrolysis oil and hemicellulosic sugars, are tested.

### **Methods**

*A. oryzae* was cultivated in preculture medium in shaking flasks for 17 hours. Due to nitrogen limitation and an excess of glucose in the production medium the fungus started to produce malic acid. A bioreactor process using glucose as carbon source was already established<sup>1</sup> and used as basis for the evaluation of alternative carbon sources. Organic acid concentration was measured by HPLC.

### **Conclusions**

*A. oryzae* proved to be a promising natural host for malic acid production with the potential to use several renewable carbon sources.

### **References:**

<sup>1</sup>Ochsenreither K, Fischer C, Neumann A, Sylдатk C (2014). Process characterization and influence of alternative carbon sources and carbon-to-nitrogen ratio on organic



acid production by *Aspergillus oryzae* DSM1863. Appl Microbiol Biotechnol. 98:5449-5460.

**FEMS-0659**

**Biotechnology and industrial microbiology**

**EXPLORING NON-CONVENTIONAL YEAST SPECIES AS SOURCE FOR  
ENHANCING ANAEROBIC XYLOSE FERMENTATION IN METABOLIC  
ENGINEERED *SACCHAROMYCES CEREVISIAE***

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**Background**

Efficient conversion of xylose, a five-carbon sugar present in significant amounts in the hemicellulose fractions of a wide range of lignocellulosic biomass, is required for the development of second generation bioethanol. Xylose is not naturally consumed by the baker's yeast, *Saccharomyces cerevisiae*, therefore heterologous pathways has to be introduced by genetic engineering. This includes introducing one of the two known pathways for the conversion of xylose to xylulose: xylose isomerase (XI) or xylose reductase and xylitol dehydrogenase (XR-XDH). In most xylose-consuming fungal species, XR is an enzyme that uses NADPH as the dominant co-factor for xylose reduction to xylitol. It has been observed that *S. cerevisiae* strains harbouring XR variants with an increased preference for NADH have more efficient xylose fermentation, therefore exploring the diversity of XRs with different co-factor affinity is essential.

**Objectives**

In this work we have evaluated the xylose utilisation under anaerobic conditions from two constructed *S. cerevisiae* strains carrying different varieties of xylose reductases.

**Methods**

The strain TMB3422, carrying a mutated version of the *Scheffersomyces stipitis* XR gene and a constructed strain, carrying a novel XR gene from a fermenting yeast species that was isolated from rotting-wood samples, were compared for characteristics under fermentative conditions.

**Conclusions**

The best xylose fermenting yeast strain analysed revealed significantly higher ethanol yield and productivity, as well as lower xylitol yield under anaerobic conditions, which represents an advance towards the efficient fermentation of lignocellulosic hydrolysates to ethanol.

**FEMS-0661**

**Biotechnology and industrial microbiology**

**SOLID WASTE FROM COFFEE PROCESS FOR  $\beta$ -GLUCOSIDASE PRODUCTION BY *BACILLUS SUBTILIS***

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**Background**

The coffee production for the Brazil is a second important *commodity* however the solid and liquid waste during the process is a environmental problem. Some works focus on use these waste to produce organic acids, enzymes and other products

**Objectives**

The aims were optimize the  $\beta$ -glicosidase production for *Bacillus subtilis* in submerged fermentation using coffee pulp as substrate and evaluated the optimize condition culture in bioreactor.

**Methods**

The coffee pulp was dried at 60 °C for 72 h until reaching constant weight. After that, the coffee pulp was ground in Willey mill (1.0 mm). The microorganism used in study was *Bacillus subtilis* CCMA0087 belongs to Culture Colection of Agricultural Microbiology (CCMA) of Federal University of Lavras (Brazil), isolated from Brazilian Cerrado fruit namely Marolo. The enzyme production was done by Response Surface methodology. The media culture was based in Kasana et al. (2008) with modification (%), 0.2 NaNO<sub>3</sub>, 0.1 K<sub>2</sub>HPO<sub>4</sub>, 0.05 MgSO<sub>4</sub>, 0.05 KCl, 0.02 peptone and coffee pulp (0.32 – 3.68) as a carbon source. The  $\beta$ -glucosidase activity as measured by p-nitrophenyl- $\beta$ -D-glucopyranoside (PNPG) as substrate.

**Conclusions**

In bench scale, *Bacillus subtilis* UFLA BCEF1130 show high  $\beta$ -glucosidase production (22.59 UI/mL) at pH 3.64, 36.6 °C and concentration coffee pulp of 36,8 g/L. However, in bioreactor the enzyme production was 2.5 less than bench scale in the same experimental conditions. The work continues to understand the scale up effect over the  $\beta$ -glucosidase production.

**FEMS-0666**

**Biotechnology and industrial microbiology**

## **CHARACTERISTIC OF EXTRACELLULAR HYALURONIDASE FROM PSEUDOZYMA APHIDIS YEAST**

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### **Background**

Hyaluronidases (Hz) are enzymes that degrade predominantly hyaluronan (HA). Until recently, three groups of Hz were reported: hyaluronate 4-glycanohydrolases (found in Vertebrates and venom of some insects), endo- $\beta$ -glucuronidases (leeches and crustaceans) and hyaluronan lyases (bacteria). Recently a new group of HA degrading enzymes from Micromycetes belonging to genus *Penicillium* was reported [Bakke M, Kamei J & Obata A., 2011, DOI: <http://dx.doi.org/10.1016/j.febslet.2010.11.021>]. These enzymes hydrolyze  $\beta$ -1,4 glycosidic bonds in HA, similar to Vertebrate Hz, however have different conserved domains. We report discovery of another producer of hyaluronate 4-glycanohydrolase from the Kingdom Fungi, the yeast *Pseudozyma aphidis*.

### **Objectives**

To characterize *P. aphidis* extracellular Hz.

### **Methods**

*P. aphidis* was cultivated in bioreactor Techfors-S with 20 L working volume (Infors AG, Switzerland). The enzyme was isolated from cultural broth filtrate and partially purified by diafiltration on Sartorius Hydra membrane cassette with 10 kDa cut-off and ion-exchange chromatography on DEAE-Sepharose Fast Flow (GE Healthcare). The products of HA enzymatic hydrolysis were analyzed by HPLC and LC-MS. Gel permeation chromatography, SDS-PAGE and Morgan-Elson colorimetric assay were used for enzyme characteristic.

### **Conclusions**

*P. aphidis* produced extracellular enzyme with hyaluronate 4-glycanohydrolase activity. Temperature and pH optima of HA hydrolysis were 45 °C and pH 3.0 respectively. The enzyme didn't hydrolyze neither chondroitin sulphate nor  $\beta$ -(1.3;1.6)-glucan schizophyllan. *P. aphidis* Hz showed high thermal stability that makes advantageous its application for enzymatic production of hyaluronan oligomers.

**FEMS-0678**

**Biotechnology and industrial microbiology**

**PENICILLIUM CHRYSOGENUM B13, A FERULOYL ESTERASE FACTORY**

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**Background**

Feruloyl esterases (FAEs) represent a diverse group of hydrolases catalyzing the cleavage of ester bonds between plant cell wall polysaccharide and phenolic acid. These enzymes are key tools for degradation of plant cell wall and are widely distributed in plants and microorganisms. FAEs release ferulic acid and other aromatic acids from these polymeric structures and have received an increasing interest in biotechnological processes for industrial and medicinal applications.

**Objectives**

In the worldwide market there are not fully purified FAEs or enough enzyme stocks. Discovery of new FAEs with novel properties continues to be an important research area that has increased drastically since 1990. Following this research line (CTM2012-32026 project<sup>1</sup>), genetic engineering techniques to improve the FAEs production in the filamentous fungus *Penicillium chrysogenum* have been applied.

**Methods**

Several transformants, whose production of FAEs is significantly improved with respect to the parental strain, have been obtained. Special interest deserves B13 transformant that multiplies the FAE activity more than 90 times when the substrate of the enzyme is methyl ferulate and over of 14 when the substrate is sugar beet pulp. Furthermore, this FAE activity from the B13 strain was compared with the activity that is present in some commercial enzymes versus synthetic and natural substrates.

**Conclusions**

The analysis of the results revealed that the B13 enzymatic extracts triplicate the FAE activity in comparison with the analyzed commercial enzymes.

<sup>1</sup>The project CTM2012-32026 is funded by Spanish Ministry of Economy and Competitiveness (MINECO) through the Subprogramme for Non-Guided Fundamental Research Projects. 2012.

**FEMS-0690**

**Biotechnology and industrial microbiology**

## **PARAMETERS AFFECTING THE PRODUCTION OF LIPASE ENZYME BY A NEW ISOLATE YEAST.**

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### **Background**

Lipases (E.C.3.1.1.3; tri glycerol acyl hydrolases) are hydrolizer enzymes which catalyze free fat into glycerol and triglycerid. Industrial lipase commonly is provided by external cellular activity from strains of microorganisms. Microbial lipases are produced in liquid phase and its activity is influenced by concentrations of carbon/ nitrogen resources, pH, temperature and metal ions.

### **Objectives**

In this study, fungi as a lipase source, isolated from the village which is located near Eskişehir city. In the production of lipase, environmental conditions and media components are investigated as important parameters.

### **Methods**

Vegetable oils as carbon source, carbohydrate source and nitrogen sources are used for lipase activity investigation. To find the optimal condition, firstly between 3.0 – 9.0 pH and 10 – 40°C several experiments are carried out to detect the effects of these varieants on lipase production.

### **Conclusions**

As a result of several experiments for production of lipase from *Rhodotorula*, the optimal condition is pH 5.0, temperature 30°C, 1% concentration of carbon from olive oil and the best source of nitrogen, the pepton has been considered. As a result of combination of carbon and nitrogen sources with olive oil, the activity of lipase was increased.

**FEMS-0693**

**Biotechnology and industrial microbiology**

**PRODUCTION OF LIPASE ENZYME BY A NOVEL FUNGAL SOURCE,  
TRICHODERMA SP.**

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**Background**

The industrial use of enzymes obtained from animals, plants and microorganisms has developed recently and the most important source for enzyme production has been determined as microorganisms. Lipases has a important application areas in industry.

**Objectives**

In this research the lipase enzyme which has a industrial importance was obtained from new fungal source that was isolated from soil. Subsequently it was determinated as *Trichoderma citrinoviride* by the analysis of 18S rRNA sequence.

**Methods**

Lipase activity was determined by titrimetric assay. Optimum conditions were detected for production of lipase.

**Conclusions**

The lipase enzyme by isolated fungi (*Trichoderma citrinoviride*) was extracellular and showed its highest activity on the 4. day of the incubation period. In the production of lipase, culture conditions and media components are investigated as important parameters. Various carbon and nitrogen sources were used for highest lipase activity. Also we detected that pH 5,5 and temperature 30 °C are optimum conditions for lipase production.



## FEMS-0705

### Biotechnology and industrial microbiology

#### MYSTERI: A ONE-STEP STEROID PRECURSORS PRODUCTION PROJECT FROM MYCOBACTERIUM TO INDUSTRY

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#### Background

The project **MySterI** (*Mycobacterial Steroids for Industry*), supported by the ERA-IB 3<sup>rd</sup> joint call, aims at producing high value steroid precursors using a novel bioconversion strategy that will yield a greening process with lower production costs (@MySterI\_ERA\_IB; <http://www.era-ib.net/mysteri-0>). The bioconversion of phytosterols (low cost plant material similar to cholesterol) to desired steroid precursors is performed using engineered fast-growing mycobacteria in a single fermentation step.

#### Objectives

The targeted precursors are: androst-4-ene-3,17-dione (AD), 3 $\beta$ -hydroxyandrost-5-ene-17-one (DHEA) and then 11 $\alpha$ -hydroxyandrost-4-ene-3,17-dione (11- $\alpha$ -OH-AD) and testosterone.

#### Methods

The key points tackled by the Consortium are: i) Genome sequencing and annotation of *Mycobacterium* sp. NRRL B-3805 (AD-producer) to identify key bioconversion genes and to enable 'omics tools; ii) Understanding of phytosterol bioconversion by means of 'omics technologies; iii) Development of the genetic engineering tools for *Mycobacterium* sp. NRRL B-3805; iv) Construction of mycobacterial strains capable of producing 11- $\alpha$ -OH-AD, DHEA and testosterone; v) Designing more efficient and eco-friendly methods of production and downstream processing for the three selected compounds

#### Conclusions

The MySterI pipeline is a top to bottom process that brings together six interdisciplinary research groups from Universities, Research Centres and Industrial companies in 3 different EU member states [Spain (**INBIOTEC**; **Gadea Biopharma**), Germany (**Technische Universität Dortmund**) and UK (**University of York**)], as

well as Norway (**Stiftelsen SINTEF**) and Russia (**Pharmins Ltd.**). Therefore, the MySterI results in progress are: i) novel strains capable of producing three valuable C19-steroid precursors from phytosterol ii) knowledge of the biochemistry of steroid biotransformations iii) optimized fermentation and eco-friendly downstream processes for the single-step production.

**FEMS-0706**

**Biotechnology and industrial microbiology**

**GENOME SEQUENCING AND 'OMICS DEVELOPMENT FOR THE STEROID  
PRECURSORS PRODUCER MYCOBACTERIUM SP. NRRL B-3805**

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**Background**

Microbial biotransformation to generate new steroid drugs is a well-established application of industrial biotechnology. Thus, several worldwide enterprises are involved in steroid production (e. g.: Gadea Biopharma SL, Sanofi, Pfizer). Biotransformation can begin with different raw materials, but the mixture of plant sterols (phytosterols) have been the research focus in recent decades. However, the studies to date show that improvements in phytosterols broth culture solubility, product recovery and mainly in microbial strains are needed.

**Objectives**

Optimization of the whole molecular biology methodologies valid for a non-well characterized microbial strain has been carried out on the androstenedione (AD)-producer strain *Mycobacterium* sp. NRRL B-3805. Thus, i) growth conditions; ii) phytosterols solubility analysis; iii) growth titration in dirty media; iv) steroids quantitation by HPLC; v) genomic DNA extraction suitable for genome sequencing; vi) high quality RNA for transcriptomics (RNAseq, microarrays) analyses; as well as vii) secreted protein purification for 2D-DIGE secretome methods have been improved.

**Methods**

An example of successful optimized process is the genome sequencing pipeline defined for *Mycobacterium* sp. NRRL B-3805. This genome sequencing and annotation, as well as the understanding of phytosterols bioconversion by means of 'omics technologies (RNAseq, microarrays, 2D-DIGE) are capital for the ERA-IB 3<sup>rd</sup> joint call supported project MySterI (@MySterI\_ERA\_IB; <http://www.era-ib.net/mysteri-0>).

**Conclusions**

Thus, key bioconversion genes and target genes suitable for the development and use of genetic engineering tools have been defined. This genome was fully sequenced using Ion Torrent and Sanger technologies. It comprises 5,421,338 bp in a circular chromosome with a GC content of 67 %.

**FEMS-0715**

**Biotechnology and industrial microbiology**

**SOLID PHASE EXTRACTION BASED ON THE USE OF HELVELLA CRISPA AS A FUNGAL BIOMASS FOR THE PRECONCENTRATIONS OF PB AND AL BY ICP-OES**

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**Background**

The use of fungal biomass for the preconcentration and separation of traces of heavy metals and some organic materials at trace levels are popular due to the good adsorption properties such as high surface area and high adsorption capacity of the fungus.

**Objectives**

This study describes the biosorption potential of *Helvella crispa* as a sorbent for solid phase extraction (SPE) and preconcentration of Pb<sup>2+</sup> and Al<sup>3+</sup>.

**Methods**

The model solution was passed through the prepared SPE column at an adjusted flow rate with a peristaltic pump. Then, 10.0 mL distilled water was passed through the column. The retained Pb<sup>2+</sup> and Al<sup>3+</sup> on the fungal biomass was then eluted with 5.0 mL of 1.0 mol L<sup>-1</sup> HCl. The concentration of Pb<sup>2+</sup> and Al<sup>3+</sup> in this solution was determined by ICP-OES.

**Conclusions**

The limit of detection (LOD) of Pb and Al was found to be 0.10 ng mL<sup>-1</sup> and 0.03 ng mL<sup>-1</sup>, respectively. The sensitivity of ICP-OES improved 39.8-fold for Pb and 39.5-fold for Al. Linearity was obtained in the concentration range of 1.25–50.0 ng mL<sup>-1</sup> for Pb and 0.5–50.0 ng mL<sup>-1</sup> for Al. The relative standard deviation (RSD) of the method under optimum conditions was lower than 8.4% (n=5) for Pb and Al, which was validated through the analysis of certified reference tea and poplar leaves samples. The biosorption capacity of immobilized *Helvella crispa* for Pb and Al was found to be 31.2 mg g<sup>-1</sup> and 45.7 mg g<sup>-1</sup>, respectively.

**FEMS-0733**

**Biotechnology and industrial microbiology**

**WHICH IS A BETTER APPROACH FOR ENZYME EXPLORATION?: CULTURE-DEPENDENT AND -INDEPENDENT (METAGENOMIC) APPROACHES TO THE SCREENING FOR AROMATIC-DEGRADING ENZYMES.**

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**Background**

Although metagenomics is regarded as one of the best approaches for mining enzymes, its superiority over traditional culture-dependent approach is still controversial.

**Objectives**

Therefore, culture-dependent and -independent approaches were used for extensive retrieval of the extradiol dioxygenase (EDO) gene from the environment to investigate the relationship between the EDO genes from isolated bacteria and the metagenomic EDO genes from which they were isolated.

**Methods**

In our previous study, we identified 91 fosmid clones showing EDO enzyme activity using a metagenomic approach (Environ Microbiol 9, 2289-2297, 2007). In the present study, we newly isolated 88 phenol-utilizing bacteria from the same environmental sample and identified four EDO genes from them. Of these, two EDOs had amino acid sequences similar to those reported previously in aromatic-utilizing strains, and one EDO had a sequence almost identical to that of metagenomic EDOs identified in our previous study. Unexpectedly, one EDO showed no similarity to any class I EDOs and was categorized as class II, which has not been found in past any metagenomic approaches. Quantitative polymerase chain reaction (PCR) assay indicated that the low-abundance class II EDO gene can be enriched by culturing approaches.

**Conclusions**

We conclude that the combined use of the two approaches can explore the gene community more extensively than their individual use.

Reference) Suenaga et al. FEMS Microbiol Ecol. 90 (2014) 367-379.

**FEMS-0741**

**Biotechnology and industrial microbiology**

## **ENGINEERING STREPTOMYCES DIASTATOCHROMOGENES 1628 TO INCREASE THE PRODUCTION OF TOYOCAMYCIN**

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### **Background**

Toyocamycin (TM) is a member of the nucleoside antibiotics. It shows antifungal activity and might be used to treat plant diseases. TM is produced by *Streptomyces diastatochromogenes* 1628 but the production rate is always very low. Recently the gene *toyG* was cloned and its function in TM biosynthesis was reported. However, nothing was known about the influence of *toyG* on TM production.

### **Objectives**

We studied the influence of three genes on TM production. *Vgb*, *frr*, and *toyG* were co-expressed, either in double- or triple-combinations.

### **Methods**

Over-expression of these genes in double or triple leads to great significant increased production of TM in strain *S. diastatochromogenes* 1628. At the end, among all these different strains constructed in this work, the strain *S. diastatochromogenes* 1628-VGF, harboring *vgb*, *frr* and *toyG* that was placed under the control of *PermE*<sup>\*</sup>, respectively, exhibited the largest increase in TM production. Notably, the TM production of *S. diastatochromogenes* 1628-VGF reached the highest level at 831.2 mg/L at 72 h, while TM yield of original strain *S. diastatochromogenes* 1628 at 415.1 mg/L at 84 h.

### **Conclusions**

Based on known knowledge, TM yield produced by engineered strain *S. diastatochromogenes* 1628-VGF was the highest as ever reported. The high-yield, energy-saving strain *S. diastatochromogenes* 1628-VGF constructed in this study shall be beneficial for the industrial production of TM.

**FEMS-0745**

**Biotechnology and industrial microbiology**

**EXPRESSION OF THE CELLULOSOMAL GENE CLUSTER OF CLOSTRIDIUM CELLULOVORANS IN BUTANOL-PRODUCING CLOSTRIDIUM BEIJERINCKII**

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**Background**

Biofuel production from cellulosic biomass consists of pretreatment, saccharification, fermentation and distillation. However, production is hindered by the high cost compared with energy crops, such that innovative technologies are required. One approach is consolidated bioprocessing (CBP), in which pretreatment, saccharification, and fermentation are consolidated.

**Objectives**

*Clostridium cellulovorans* 743B, an anaerobic and mesophilic bacterium, produces a large extracellular enzyme complex (cellulosome) that efficiently hydrolyzes cellulosic biomass. The cellulosomal gene cluster of *C. cellulovorans* includes *cbpA*, which encodes a scaffold protein, and seven downstream glycoside hydrolase genes. *Clostridium beijerinckii* NCIMB 8052 cannot produce a cellulosome and does not degrade soft biomass, but instead produces butanol, acetone, and ethanol from mono- and disaccharides. The aim of this study was to express the cellulosome of *C. cellulovorans* in *C. beijerinckii* to obtain a bacterium able to carry out CBP.

**Methods**

The large cellulosomal gene cluster containing the *cbpA* gene was used to transform *C. beijerinckii* by electroporation. Protein-level expression of the gene cluster encoding the scaffold protein CbpA and mannanase A was demonstrated by western blotting. Cellulose and galactomannan activities on polysaccharide substrates were evaluated by Congo red staining.

**Conclusions**

The transformant was shown to be capable of hydrolyzing galactomannan and produced butanol directly. This butanol-producing bacterium provides the basis for the CBP of lignocellulose to biobutanol.

**FEMS-0756**

**Biotechnology and industrial microbiology**

## **EFFECTS OF IRON AND MAGNESIUM IONS COMBINATION ON HYDROGEN PHOTOPRODUCTION BY RHODOBACTER SPHAEROIDES**

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### **Background**

Iron and magnesium are essential for growth of photosynthetic bacteria.  $Mg^{2+}$  participates in the structure of photosynthetic electron carriers and pigments.  $Fe^{2+}$  is known as the component of enzymes, involved in bio-hydrogen ( $H_2$ ) production.

### **Objectives**

In this work the effects of metal ions combinations on growth and  $H_2$  photoproduction by *Rhodobacter sphaeroides* MDC6522, isolated from Jermuk mineral springs in Armenia, in compare to single ions effects, were investigated.

### **Methods**

*R. sphaeroides* was cultivated under anaerobic conditions upon illumination in the presence of  $Fe^{2+}$  and  $Mg^{2+}$  various concentrations. The  $H_2$  yield was calculated by the decrease of redox potential, measured by platinum electrode.

### **Conclusions**

The results pointed out the concentration dependent effects of metal ions. *R. sphaeroides* was unable to grow well in the absence of  $Fe^{2+}$  and  $Mg^{2+}$ , which indicates the importance of these ions for bacterial growth. The highest growth rates of *R. sphaeroides* cells were obtained in the presence of 0.08 mM  $Fe^{2+}$  and 5 mM  $Mg^{2+}$ , in compare to control (0.04 mM  $Fe^{2+}$  and 1 mM  $Mg^{2+}$ ). The simultaneous addition of metal ions stimulated bacterial growth rate ~1.2-fold in compare to the single ions. The highest  $H_2$  yields were obtained in the presence of 0.08 mM  $Fe^{2+}$  and 10 mM  $Mg^{2+}$ , which were ~1.5-fold higher than control. However, the  $H_2$  production was increased ~2.5-fold, when two metals were added simultaneously in concentration 0.08 mM  $Fe^{2+}$  and 5 mM  $Mg^{2+}$ . Thus, combinations of metal ions in appropriate concentrations are preferable for  $H_2$  production enhancement in *R. sphaeroides*.



**FEMS-0764**

**Biotechnology and industrial microbiology**

**HIGH-LEVEL EXPRESSION, RAPID ON-COLUMN REFOLDING AND  
PURIFICATION OF RECOMBINANT HUMAN VASCULAR ENDOTHELIAL  
GROWTH FACTOR 165 IN ESCHERICHIA COLI**

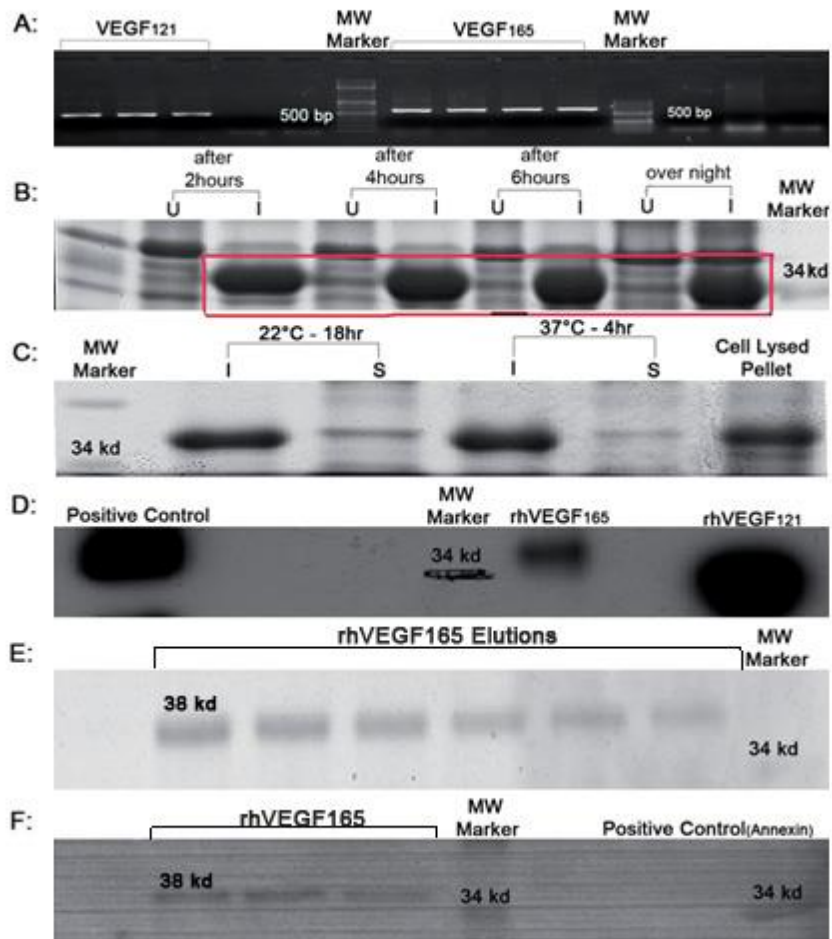
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**Background**

Vascular endothelial growth factors (VEGFs) are a family of proteins that promote angiogenesis and participate in a variety of physiological and pathological processes. VEGF165 is the major isoform induced by hypoxia, oncogene mutations, and cytokines such as IL-1, IL-8, and TNF- $\alpha$  and secreted by many cell types. It is a potent angiogenic factor and mitogen that stimulates proliferation, migration, and formation of endothelial cells. Cancerous cells are able to grow and metastasize by VEGF secretion.

**Objectives**



A. PCR screening of the recombinant plasmid pET32a+ ( VEGF165).

B. SDS-PAGE analysis of rhVEGF165 expression in BL21(DE3) cells  
 U: uninduction (-IPTG)  
 I: Induction (+IPTG) restriction sites.

C. The effects of induction time and temperature on soluble and insoluble rhVEGF165 aftr cell disruption  
 I: Insoluble  
 S: Soluble.

D. SDS-PAGE analysis and immunoblot with anti-His-tag antibody .

E. SDS-PAGE analysis of purified rhVEGF165 with On-Column refolding system.

F. SDS-PAGE and Western Blot analysis of purified rhVEGF165 using anti-His-tag antibody.

A recombinant VEGF165 might be used in order to produce an antibody against it and also it could be used as a growth factor in some biological mediums.

## Methods

The RNA was extracted from Hela cell, and then used for cDNA synthesis. The gene encoding human VEGF isoform 165 (hVEGF165) was cloned into the expression vector pET32a (+) to construct a fusion expression plasmid that induced the thioredoxin (Trx) Gene. The expressed protein was purified by affinity chromatography using Ni-NTA resin. High concentration of the recombinant protein obtained from a single-step.

## **Conclusions**

Fusion of VEGF to thioredoxin and careful codon optimization of the eukaryotic sequence could be improved to a high-level insoluble protein expression in comparison to an un-optimized, His-tagged construct. The thioredoxin-fused protein was successfully purified using an on-column Ni-NTA purification procedure. SDS-PAGE and Western Blotting analysis confirmed the desired expression. rhVEGF165 could readily and rapidly purify with on-column Ni-NTA system chromatography.

FEMS-0793

Biotechnology and industrial microbiology

## ADHESION OF ALKANOTROPHIC ACTINOBACTERIA TO SOLID SURFACES AND HYDROPHOBIC LIQUIDS

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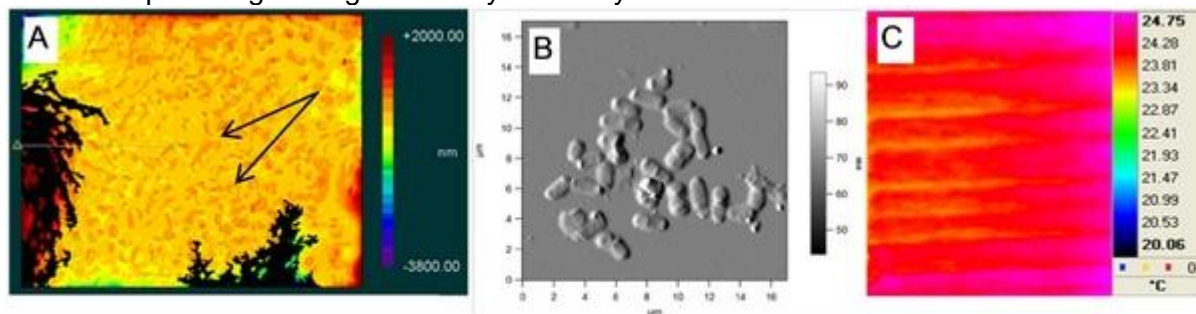
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### Background

Mechanisms of adhesion of biotechnologically significant alkanotrophic actinobacteria are poorly studied.

### Objectives

1. To study the adhesion of actinobacteria to a wide range of solid carriers and hydrophobic liquids under various growth conditions. 2. To determine physical–chemical and molecular factors of actinobacterial adhesion. 3. To define adhesion conditions providing the highest catalytic activity of actinobacteria.



**Figure.** Images of monolayer actinobacterial biofilms obtained with an interference microscope (A), an atomic force microscope (B), and an infrared camera (C). Arrows show cells.

### Methods

100 strains from the IEGM Collection of Alkanotrophic Microorganisms (acronym IEGM, WDCM # 768, [www.iegml.ru/iegmlcol](http://www.iegml.ru/iegmlcol)) were used in this study. The substrates for adhesion were polystyrene microplates, technical polymer tissue, industrial wastes (sawdust, poultry feathers, and leather), *n*-alkanes C10–C16, solvents, and different oils. Molecular genetic and biochemical mechanisms of adhesion were studied using targeted and *in vivo* Tn5 mutagenesis, chemical analysis of cell wall, and specific inhibition. Cell morphology, nanomechanical properties, adhesion forces, and distribution of actinobacteria on solid carriers were studied by interference microscopy and combined confocal and laser scanning atomic force microscopy. Thermodynamic data were obtained using infrared thermography.

### Conclusions

Adhesion was shown to be a key mechanism in hydrophobic compound oxidation by

actinobacteria. Involvement of glycolipid biosurfactants, proteins, and cell appendages in adhesion was revealed. Monolayer actinobacterial biofilms with high ( $115 \text{ mg} \cdot \text{l}^{-1} \cdot \text{h}^{-1}$ ) hydrocarbon–oxidizing activities were formed on solid surfaces hydrophobized with glycolipid biosurfactants (Figure). The results obtained could be used to develop biocatalysts for targeted biotransformations and pollutant degradation. The research was supported by the RFBR grant (14-04-96013), the RSF grant (14-14-00643), and the President of the Russian Federation for Leading Scientific Schools' grant (4607.2014.4).

**FEMS-0796**

**Biotechnology and industrial microbiology**

## **BIOTRANSFORMATION OF BETULIN USING RHODOCOCCUS ACTINOBACTERIA**

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### **Background**

Bacterial transformation of triterpenoids (in particular betulin) is an efficient method for single-stage production of biologically active compounds with high regio- and stereoselectivity.

### **Objectives**

1. To demonstrate the possible use of *Rhodococcus* actinobacteria for selective biotransformation of betulin, a pentacyclic lupine-type triterpenoid. 2. To study the interaction mechanisms between rhodococci and betulin, and its biotransformation pathways. 3. To evaluate the possible application of products from bacterial betulin oxidation for subsequent synthesis of biologically active compounds using chemical methods.

### **Methods**

The biological resources of the IEGM Collection of Alkanotrophic Microorganisms (acronym IEGM, WDCM # 768, [www.iegm.ru/iegmcol](http://www.iegm.ru/iegmcol)) were used in this study. Conventional bacteriological methods, including betulin transformation by resting rhodococcal cells were employed. The mechanism of cell interaction with betulin was determined using methods of phase contrast, confocal lazer scanning, and atomic force microcopy. Rhodococcal viability was estimated using a LIVE/DEAD® BacLight™ Bacterial Viability Kit (Invitrogen, USA).

### **Conclusions**

Biotransformation of betulin (0.5 and 3.0 g/l) using *Rhodococcus* actinobacteria was demonstrated for the first time. Betulone was identified as a major metabolite. The optimal conditions for betulin bioconversion by resting cells of *R. rhodochrous* IEGM 66 were selected. Further chemical modifications of betulone resulting from bacterial betulin transformation lead to the formation of 3,4-secobetulone with a marked cytotoxic activity (IC<sub>50</sub> 3.05 мкМ) against melanoma.

The research was supported by the RFBR grant (14-04-96017), and the President of the Russian Federation for Leading Scientific Schools' grant (4607.2014.4).

**FEMS-0808**

**Biotechnology and industrial microbiology**

**COMMERCIALLY IMPORTANT HIGH MOLECULAR WEIGHT DEXTRAN FROM INDIGENOUS WEISSELLA CIBARIA CMGDEX3**

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**Background**

Extracellular polysaccharides (EPS) of microbial origin are associated with the cell surface or excreted in the extracellular environment. Several food grade microorganisms, including lactic acid bacteria (LAB), synthesize EPS. The synthesis of EPS by LAB has gained remarkable interest because of LAB categorization as safe organisms and vast utilization of synthesized EPS in markets from food to health and industry. Dextran is a polysaccharide composed of D-glucose units which is synthesized by various LAB under appropriate conditions and widely used in several industries including food, pharmaceutical and chemical.

**Objectives**

Present study was designed to isolate LAB capable to synthesize good quality dextran and characterize the synthesized dextran.

**Methods**

In this study five bacterial strains were isolated from different vegetables and fruit samples on the basis of slime production in selected agar medium with 15% sucrose. When those five strains were cultivated in medium for the production and extraction of EPS, the highest crude yield of EPS was obtained from CMGDEX3 which was isolated from cabbage and identified as *Weissella cibaria* by 16S rRNA gene sequencing. EPS extracted from *W. cibaria* CMGDEX3 was purified and analyzed for structure by FTIR, <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy. Molecular weight was determined by gel permeation chromatography.

**Conclusions**

Analysis of EPS demonstrated that *W. cibaria* CMGDEX3 synthesized commercially important linear dextran that predominately had α (1→6) glycosidic linkages with few (3.4%) α (1→3) linked branches. Molecular mass determination showed that it was a high molecular weight dextran of an average > 2,000,000 Daltons.

**FEMS-0815**

**Biotechnology and industrial microbiology**

**SCREENING AND SELECTION OF PROBIOTIC LACTIC ACID BACTERIA  
INHIBITING PATHOGENIC BACTERIA AND VIRUSES**

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**Background**

Probiotic refers to viable, nonpathogenic microorganisms that, when ingested, are able to reach the intestines in sufficient numbers to confer health benefits to the host. Intake of probiotics reduces the amount of pathogens improving thus the intestinal microbial balance of the host and lowering the risk of gastro-intestinal diseases.

**Objectives**

To isolate potential probiotic bacterial strains inhibiting rota- and polio- viruses as well as pathogenic bacteria.

**Methods**

Three thousands bacterial strains isolated from various sources including human milk and fermented foods were screened for potential probiotics inhibiting rota- and polio-viruses as well as pathogenic bacteria such as enterotoxigenic *Escherichia coli*, *Clostridium perfringens*, *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella* Typhimurium,. The antibacterial activity of each culture filtrate was determined by agar-diffusion method and the antiviral activity of heat-treated culture broth was examined by reduction of plaque forming unit *in vitro* culture system. The properties of the bacterial strains with particular functions such as adherent ability to enterocyte, tolerances towards gastric juice, bile, and tolerances to heat and cold-dry conditions were further investigated to select strains for probiotic use.

**Conclusions**

One potential lactic acid bacterium with desirable probiotic characteristics as well as the highest anti-viral and anti-bacterial activities was selected and identified as *Lactobacillus plantarum* according to its 16rRNA gene sequence.



**FEMS-0830**

**Biotechnology and industrial microbiology**

## **OPTIMIZATION OF THE SECRETION OF BACTERIOCINS AND THE BACTERIAL CELL GROWTH OF *GEOBACILLUS* SP. 15 STRAIN**

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### **Background**

Bacteriocins are natural peptides secreted by variety of bacteria and provide the bacteria with a competitive advantage in their environment, eliminating competitors to gain resources. Bacterocin production may be strongly influenced by growth environment and medium composition. Many different studies have indicated the potential usefulness of bacteriocins in food preservation, veterinary, medicine or as phytosanitary measures for plant protection. To date, only few bacteriocin producing thermophilic bacterial strains of genus *Geobacillus* have been identified. Studies of factors affecting the production of bacteriocins by *Geobacillus* spp. bacteria are relatively scarce.

### **Objectives**

This research was focused on the analysis effect of salts and to establish optimal conditions for bacteriocins production and bacterial cell growth for thermophilic bacteria *Geobacillus* sp. 15 strain.

### **Methods**

*Geobacillus* sp. 15 strain was used as a bacteriocin producer. *Geobacillus stearothermophilus* NUB36187 (BGSC No. 9A11) was selected as the indicator strain for the bacteriocin assay. Nutrient broth (NB) liquid media were chosen for the strain growth and bacteriocin expression monitoring. It were supplemented with salts: KCl, ZnSO<sub>4</sub>, MnSO<sub>4</sub>, CuSO<sub>4</sub>, MgCl<sub>2</sub>, CaCl<sub>2</sub>, NaNO<sub>3</sub>, NaHCO<sub>3</sub>. Bacteriocin activity was detected by agar well diffusion assay.

### **Conclusions**

The greatest influence on bacterial growth was in NB medium with MnSO<sub>4</sub> or with KCl and MgSO<sub>4</sub>. The best bacteriocin expression was achieved in media supplemented with two salts at a time: KCl and MgCl<sub>2</sub>, MnSO<sub>4</sub> and NaHCO<sub>3</sub> or CaCl<sub>2</sub> and ZnSO<sub>4</sub>.

**FEMS-0838**

**Biotechnology and industrial microbiology**

**METABOLIC ENGINEERING OF *C. GLUTAMICUM* THROUGH CONSOLIDATED BIOPROCESSING FOR SUCCINATE PRODUCTION FROM CO<sub>2</sub>-GROWN MICROALGAL BIOMASS AS CARBON SOURCE**

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**Background**

This study presents the development of consolidated bioprocessing for succinate production from microalgal biomass using engineered *Corynebacterium glutamicum*.

**Objectives**

Our objective in this study is to develop the *C. glutamicum* strain capable of utilizing starch as carbon source using the synthetic biology-platform and to apply the strain for production of succinate from microalgal biomass as sole carbon source.

**Methods**

We developed the starch-degrading and succinate-producing *C. glutamicum* strains, which produced succinate (0.16 g succinate/g total carbon source) from a mixture of starch and glucose as a model microalgal biomass. Subsequently, the engineered *C. glutamicum* strains were able to produce succinate (0.28 g succinate/g of total sugars) from pretreated microalgal biomass of CO<sub>2</sub>-grown *Chlamydomonas reinhardtii*. For the first time, this work shows succinate production from CO<sub>2</sub> via sequential fermentations of CO<sub>2</sub>-grown microalgae and engineered *C. glutamicum*.

**Conclusions**

In conclusions, consolidated bioprocessing based on microalgal biomass could be useful to promote variety of biorefineries.

**FEMS-0854**

**Biotechnology and industrial microbiology**

**AQUEOUS TWO PHASE EXTRACTION OF JONESIA DENITRIFICANS  
XYLANASE 6 IN PEG 1000/PHOSPHATE SYSTEM.**

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**Background**

The impetus for research in the field of bioseparation has been sparked by the difficulty and complexity in the downstream processing of biological products. Indeed, 50% to 90% of the production cost for a typical biological products resides in the purification strategy. There is a need for efficient and economical large scale bioseparation techniques which will achieve high purity and high recovery, while maintaining the biological activity of the molecule. One such purification technique which meets these criteria involves the partitioning of biomolecules between two immiscible phases in an aqueous system (ATPS).

**Objectives**

The purification of xylanase from the strain *Jonesia denitrificans* BN13 using ATPS an efficient and economical bioseparation technique.

**Methods**

The Production of xylanases is carried out in 500ml of a liquid medium containing birchwood xylan. In each ATPS, PEG 1000 is added to a mixture consisting of dipotassium phosphate, sodium chloride and the culture medium inoculated with the strain *Jonesia denitrificans*. The concentration of PEG 1000 was varied : 8 to 16 % and the NaCl percentages are also varied from 2 to 4% while maintaining the other parameters constant, the xylanolytic activity was detected by zymogram coupled to SDS-PAGE.

**Conclusions**

The results showed that the best ATPS for purification of xylanases is composed of PEG 1000 at 8.33%, 13.14 % of  $K_2HPO_4$  , 1.62% NaCl at pH 7. We obtained a yield of 96.62 %, a partition coefficient of 86.66 and a purification factor of 2.9. The zymogram showed that the activity is mainly detected in the top phase.

**FEMS-0859**

**Biotechnology and industrial microbiology**

**PROTEOMICS TO ELUCIDATE THE MOLECULAR PHYSIOLOGY OF MICROBISPORA SP. ATCC-PTA-5024, THE PRODUCER OF NAI-107, A VERY PROMISING LANTIBIOTIC**

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**Background**

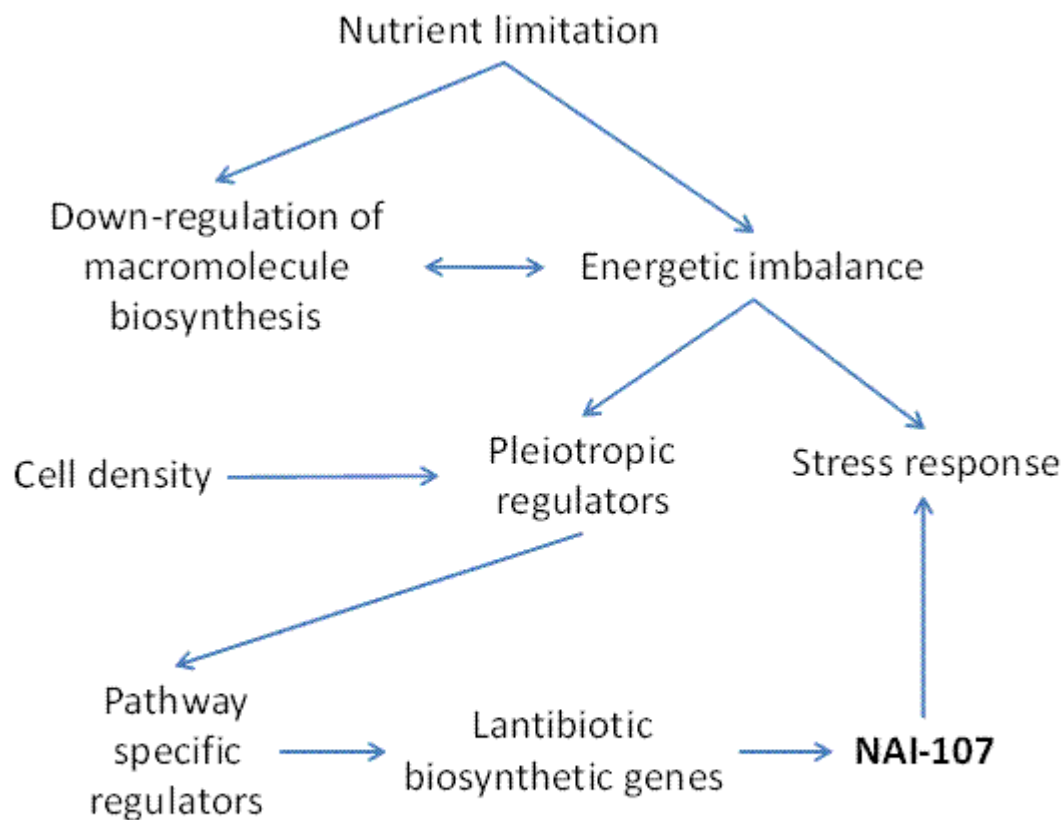
The filamentous actinobacterium *Microbispora* sp. ATCC-PTA-5024 produces the lantibiotic NAI-107 (Maffioli *et al.*, 2014), which is effective against multidrug-resistant Gram-positive pathogens (Jabés *et al.*, 2011).

In actinomycetes, the biosynthesis of antibiotics is generally elicited as a physiological response controlled by a complex regulatory network involving global regulators, playing pleiotropic roles, and pathway-specific regulators, which activate the biosynthesis of biologically active molecules (Bibb, 2005).

**Objectives**

The integration of bioinformatic tools and holistic technologies has allowed the development of consolidated strategies to manage huge amounts of complex molecular information on gene expression and biochemical capabilities deriving from 'omic' investigations, ultimately leading to novel approaches to explore microbial physiology. In the perspective of using *Microbispora* strains for the industrial synthesis of a NAI-107, insights on the molecular physiology of this actinobacterium would be beneficial to develop robust and economically-feasible production

processes.



**Figure 1.** Synoptic scheme summarizing the signalling systems and the regulatory cascades associated with the physiological differentiation processes in *Microbispora* sp.

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## Methods

An extensive investigation on proteomic changes associated with lantibiotic production was performed on the *Microbispora* sp. ATCC-PTA-5024 wild type strain at different growth stages by using combined two-dimensional difference in gel electrophoresis and mass spectrometry approaches. To evaluate the effect of NAI-107 on bacterial vitality, comparative proteomic experiments were also performed on a not producing strain following NAI-107 addition. The obtained results were then integrated with bioinformatics, fluorescence microscopy and molecular genetic experiments.

## Conclusions

The results of this study elucidate the regulatory networks, the biochemical pathways and the molecular processes occurring during growth and lantibiotic production (Figure 1), thus providing the first functional picture of a member of the *Microbispora* genus.



**FEMS-0878**

**Biotechnology and industrial microbiology**

**OPTIMISATION OF THE BIOFLOCCULANT PRODUCED BY PANTOEAL SP., A NOVEL BACTERIUM ISOLATED FROM SEDIMENT FROM THE BEACH AT MTUNZINI, KWAZULU-NATAL, SOUTH AFRICA**

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**Background**

We screened microorganisms with potential for bioflocculation. An organism that secretes large amounts of bioflocculant was isolated from a sediment sample collected from the beach at Mtunzini on the coast of northern Kwazulu-Natal, South Africa

**Objectives**

The primary objective of this study was to optimised the bioflocculant production by the *Pantoea* sp. Further studies on the characterisation and applications of the bioflocculant produced by this organism will be done to underscore the biotechnological importance of this species as a producer of secondary metabolite useful for water and wastewater treatment

**Methods**

From detailed morphological and biochemical analysis, the isolate was identified as *Pantoea* sp. with 91% probability. Cultivation studies revealed that the isolate produced bioflocculant optimally with sucrose as a source of carbon (92.4% flocculating activity), inoculum size of 3% (v/v), at an initial pH of 6.0, with Ca<sup>2+</sup> as an aid to coagulation, and a production time of 96 h

**Conclusions**

We proposed that this organism has great potential as a producer of bioflocculant due to its ability of producing high flocculating activity when cultivation conditions are varied.

**FEMS-0883**

**Biotechnology and industrial microbiology**

**INFLUENCE OF THE MOLASSES AND AERATION ON THE PRODUCTION OF CELLULOSE BY GLUCONACETOBACTER XYLINUS**

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**Background**

The bacterial cellulose (BC), produced by *Gluconacetobacter xylinus*, has been presented as a useful alternative to replace the vegetable cellulose as well as their use in many industrial applications. A carbon source and a system optimized for efficient aeration is important for a greater production of BC.

**Objectives**

This study has as its objective determine the effect of molasses in the production of BC by *Gluconacetobacter xylinus* IFO 13693 under static conditions and static with aeration.

**Methods**

The synthesis of bacterial cellulose (BC) by *Gluconacetobacter xylinus* was carried out in static culture discontinuous with and without aeration intermittent at room temperature, in the presence of molasses as the main carbon source at initial concentrations of 13.3 % (w/v). The concentration of BC concentrations, sucrose, glucose and fructose were determined each week. To determine the formation of cellulose and the coefficient of performance of the product was used the Microcal Origin software 6.0®.

**Conclusions**

By the fourth week, BC values found in the static and static and aeration experiments were around 103 g/L and for static 78.7 g/L, respectively. The kinetics for the hydrolysis of sucrose in the medium fixed the model of Michaelis-Menten, with a  $V_{max}$  of 0.0041 moles/L/h and 0.0036 moles/L/h, and a  $K_m$  of 0.021 M and 0.019 M for each culture condition, respectively. The yield coefficients have values of 0.013 to 8.7 g dry cellulose/g of glucose consumed. This shows the importance of the substrate and aeration on the synthesis of cellulose.



**FEMS-0886**

**Biotechnology and industrial microbiology**

## **SCREENING OF FUNGI ISOLATED FROM SELECTED WASTE FOR LACCASE PRODUCTION**

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### **Background**

Laccases are polyphenol oxidases with numerous industrial and bioremediation application, most laccase activity is demonstrated in fungal species.

### **Objectives**

The study evaluated the potential of fungi isolated from selected agro waste for laccase production

### **Methods**

Fungi cultures were screened for laccase production by plate test using 2, 2, azinobis ( 3 ethyl benthiazoline 6 sulphonate) (ABTS) and submerged culture using synthetic medium.

### **Conclusions**

Utilisation of selected agro wastes (sawdust, plantain and banana peel) residue for laccase production was evaluated. Five out of twelve isolates were positive and identified as *GEOTRICUM* Spp, *CEPHALOSPORIUM* Spp, *TRICHODERMA* Spp, *TRAMETES* Spp and *FUSARIUM* Spp. Optimum enzyme activity was observed using *TRICHODERMA* Spp at 57.1U/ml followed by *TRAMETES* Spp with 51.99U/ml, *FUSARIUM* Spp had 29.2U/ml while *GEOTRICUM* Spp and *CEPHALOSPORIUM* Spp had 28.04U/ml and 9.72U/ml respectively. *TRICHODERMA* Spp was selected as the most potent in producing enzyme and therefore used for further studies. Effect of carbon source, inoculum sizes, pH and shaker speed was evaluated. Sawdust gave the highest yield with 151.17U/ml followed by plantain peel with 62.49U/ml, banana peel was least with 54.94U/ml. The optimum pH for sawdust, plantain peel and banana peel was 5.95, 5.94 and 5.83 respectively. using shaker incubator @ 150rpm with sawdust as carbon source, Laccase yield of 310U/ml was obtained at pH of 6 and temperature of 25 degree celcius inoculum size of 1000000 spores per ml. Thus sawdust can serve as cheap substrate for laccase production

**FEMS-0936**

**Biotechnology and industrial microbiology**

**IDENTIFICATION OF CELLULOSOMAL ENZYMES FOR THE CONSTRUCTION OF CELLULOSOMES FROM CLOSTRIDIUM CELLULOVORANS**

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**Background**

*Clostridium cellulovorans* 743B, an anaerobic mesophile, produces a multienzyme complex, called the cellulosome, for the efficient degradation of cellulosic biomass. The cellulosome consists of carbohydrate enzymes and scaffolding proteins. The scaffolding protein CbpA consists of nine hydrophobic repeat domains (cohesin domains) for the binding of enzymes containing dockerin domains (cellulosomal enzymes).

**Objectives**

The expression pattern of the cellulosomal enzymes in the presence of various carbon sources was previously shown by proteome analysis, but the constituent enzymes of the cellulosome and their arrangement as a complex have yet to be determined. In this study, we identified the cellulosomal enzymes involved in the cohesin-dockerin interaction.

**Methods**

*C. cellulovorans* was cultivated on cellobiose and its cellulosome was purified from cell extracts using Avicel affinity chromatography. Fourteen enzymes were found to make up the cellulosome. Nine glutathione-S-transferase (GST) fusion mini-scaffolding proteins, each containing a cohesin domain of CbpA, were expressed in *Escherichia coli*. Cellulosomal enzymes that specifically or nonspecifically bound cohesin domains were detected by GST pull-down assay, which was also used to qualitatively analyze the interaction between five types of dockerin domains and all cohesin domains.

**Conclusions**

The interaction was found to be significantly dependent on the type of dockerin domain and the particular cohesin domain. These results imply a specific arrangement of enzymes in the cellulosome.



**FEMS-0951**

**Biotechnology and industrial microbiology**

## **TOWARDS BIOTECHNOLOGICAL APPLICATION OF PSEUDOMONAS SPP. LIPASES**

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### **Background**

Microbial lipases catalyze hydrolysis of lipids, enabling bacteria to use vegetable and other oils as a carbon and energy source. Lipid hydrolysis share common intermediates with synthesis of valuable biopolymer polyhydroxyalkanoate (PHA). Bringing together these two processes in a single microorganism could further increase its potential for application in industrial biotechnology.

### **Objectives**

The objective was to screen *Pseudomonas* strains for lipase activity and capability to accumulate PHA in order to identify or to genetically modify these microorganisms to use waste cooking oils for production of valuable biopolymers.

### **Methods**

Seven *Pseudomonas* strains were tested for lipase activity on agar plates containing either Tween 80 or olive oil as carbon source. Bacterial capability to utilize reused fryer oil as a sole carbon source was tested in liquid medium. For selected *Pseudomonas* strains lipase activity was quantified using para-nitrophenyl palmitate enzyme assay and genetic determinants of lipase activity were amplified. In order to select appropriate host for the heterologous expression of lipase all *Pseudomonas* strains were tested for their ability to accumulate PHA.

### **Conclusions**

Among seven tested *Pseudomonas* strains *Pseudomonas aeruginosa* PAO1 showed highest lipase activity, while both *P. aeruginosa* PAO1 and *Pseudomonas putida* KT2440 could accumulate PHA. Since *P. aeruginosa* PAO1 is known opportunistic pathogen, *P. putida* KT2440 was more appropriate host for the biopolymer

production. *P. aeruginosa* PAO1 lipase-encoding genes were amplified and cloned into *P. putida* KT2440, and heterologous expression was optimized. Further analyses should reveal if these constructs have potential for the industrial application.

**FEMS-0952**

**Biotechnology and industrial microbiology**

## **MYSTERI: MYCOBACTERIAL STEROIDS FOR INDUSTRY**

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### **Background**

Steroid based pharmaceuticals are extremely important for health, and conversion of cheap agricultural waste into highly valuable steroids is therefore an attractive prospect. Previous studies have indicated that in order to achieve this several areas need improvement, including the microbial strains used for bioconversion.

### **Objectives**

By utilizing the bacterium *Mycobacterium* sp. NRRL B-3805 we aim to replace the current microbial and chemical multistep processes with a single-step production of several steroids including androstenedione (**AD**), 11 $\alpha$ -hydroxy-androstenedione (**11 $\alpha$ -OH-AD**), dehydroepiandrosterone (**DHEA**) and **testosterone** from a cheap phytosterol feedstock.

### **Methods**

We have developed genetic engineering tools and procedures to manipulate *Mycobacterium* sp. NRRL B-3805 to improve the bioconversion of phytosterols. *Mycobacterium* sp. NRRL B-3805 metabolises steroids through the action of cholesterol oxidases, which modify the A-ring of the steroid core before cleaving the side chain through multiple enzymatic steps to produce **AD**. The genome of *Mycobacterium* sp. NRRL B-3805 was sequenced and was mined for potential cholesterol oxidases, hydroxysteroid dehydrogenases and other known enzymes involved in cholesterol catabolism. The genome mining enabled strain manipulation, as a means to optimise steroid production. Quantitation of steroid production was determined using GCMS.

### **Conclusions**

Production of **11 $\alpha$ -OH-AD** is being investigated by amplifying a putative 11 $\alpha$ -hydroxylase and the associated oxidoreductase from *Aspergillus ochraceus* cDNA and placing both coding sequences under bidirectional inducible promoters for expression in *Saccharomyces cerevisiae* and **11 $\alpha$ -OH-AD** production in media

containing **AD**. If active these enzymes will be expressed in *Mycobacterium* sp. NRRL B-3805.

**FEMS-0960**

**Biotechnology and industrial microbiology**

## **BIOCALORIMETRY – AN EFFICIENT TOOL TO CONTROL MICROBIAL GROWTH AND PRODUCT FORMATION ON TOXIC FEEDSTOCKS**

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### **Background**

In the future, petrochemical industry may be replaced by biotechnological processes producing building block chemicals from renewable carbon and employing genetically engineered bacteria and other microorganisms. However, besides searching for suitable microbial production platforms, a major hurdle for a large-scale implementation of bioprocess-based technologies is the availability of economic and sustainable carbon sources.

### **Objectives**

An alternative substrate not competing with food or fodder production might be methanol due to its high synthesis capacities and a potentially sustainable production from natural gas, agricultural waste materials and biogas. However, main weaknesses of this C1 compound are its high volatility and toxicity. Its use in fermentation processes therefore calls for an innovative real-time control strategy.

### **Methods**

In this study, we decided on biocalorimetry as a basis for optimizing C1 substrate feed regimes in fermentations with the methylotrophic bacterium *Methylobacterium extorquens*. According to Hess' law every metabolic flux is quantitatively related to the heat production rate. Thus, calorimetry provides real-time stoichiometric and kinetic information of the fermentation process and allows reacting fast and efficiently to any metabolic changes.

### **Conclusions**

By comparing different calorimetry-based control strategies we established a procedure leading to the highest growth and product formation rates of *M. extorquens* on methanol. Additionally, we could show that this approach can be extended to other, even more toxic substrates such as formic acid. In conclusion, this study demonstrates that biocalorimetry is a very efficient tool for process control and can be applied to optimize any microbial product formation process.





**FEMS-0963**

**Biotechnology and industrial microbiology**

**PHYSICOCHEMICAL AND BIOLOGICAL PROPERTIES OF THE ANTIFUNGAL  
POLYENE ANTIBIOTIC ROSEOFUNGIN**

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**Background**

Due to the upward trend of fungal infections and development of resistance to existing drugs, the requirement for efficient antifungal agents has increased.

**Objectives**

Strain *Streptomyces roseoflavus* var. *roseofungini* 1-68, a producer of the antibiotic roseofungin.

**Methods**

Methods for spectrometry, NMR- and mass spectroscopy, elemental analysis, ozonolysis, and biological testing were used.

**Conclusions**

Roseofungin is an original natural highly efficient antifungal antibiotic. Roseofungin is amorphous powder, it decomposes at  $t > 130^{\circ}\text{C}$ ; fluoresces in UV, is quite soluble in pyridine, dimethylformamide, acetic acid, lower alcohols, soluble in water-saturated butanol and water acetone. UV-spectrum has 2 absorption maxima: at 263 nm and 363 nm. Roseofungin in concentrations of 0.5-12.5  $\mu\text{g/ml}$  inhibits the pathogenic agents of superficial and deep mycoses-trichophytosis, microsporia, candidiasis, cryptococcosis, sporotrichosis, chromomycosis, aspergillosis, and others. High virus-inhibiting activity of roseofungin has been established against various strains of influenza virus, Sendai paramyxovirus and Newcastle disease virus.

**FEMS-0975**

**Biotechnology and industrial microbiology**

**DEVELOPMENT OF NEW BIOLOGICAL PREPARATIONS OF THE “RIZOVIT-AKS” SERIES BASED ON NODULE BACTERIA OF LEGUMINOUS PLANTS CULTIVATED IN KAZAKHSTAN**

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**Background**

Development of biological preparations based on active strains of indigenous nodule bacteria of leguminous plants improves their productivity and soil capabilities.

**Objectives**

Strains of nodule bacteria isolated from the rhizosphere of leguminous plants: *Bradyrhizobium japonicum* M-1 from soybean, *Shinorhizobium meliloti* L5-1 from alfalfa, *Rhizobium leguminosarum* G2 from peas, *Mesorhizobium cicer* U-2O from chickpeas, *Rhizobium leguminosarum* B-1 from lentils.

**Methods**

Biotechnological and microbiological methods were used in the study.

**Conclusions**

Modification of the culture media containing bean broth or yeast extract has been carried out, and effect of carbon and inorganic salt sources on the growth and biomass accumulation of indigenous strains of nodule bacteria from soybean, alfalfa, peas, chickpeas, lentils studied. The best carbon source for most strains of root nodule bacteria is sucrose at a concentration of 4-10.0 g/L. A number of salts (CaCO<sub>3</sub>, MnSO<sub>4</sub>, ZnSO<sub>4</sub>, CoCl<sub>2</sub>) stimulated the biomass accumulation of nodule bacteria strains up to 10<sup>11</sup> CFU/ml.

Application of biological preparations of the “Rizovit-AKS” series to inoculate the seeds of leguminous crops reduces the time required for passing phenophases by legumes: on average 2 to 3 days for soybean; 2 to 5 days for peas; up to 2 days for lentils, 2 to 4 days for alfalfa. Biopreparations increase the plant growth, green mass and number of nodules under all tested soil and climatic conditions of Kazakhstan. With the use of biopreparations, an increase in the yields of leguminous crops: for soybeans by 3.2 t/ha (34.4%), lentils – by 3.3 t/ha (41.3%); peas – by 8.3 t/ha (140.7%).

**FEMS-0980**

**Biotechnology and industrial microbiology**

**FROM ORGANIC WASTE TO RENEWABLE ENERGY: A NOVEL HYBRID DARK FERMENTATION-PHOTOHETEROTROPHIC BIOHYDROGEN PRODUCTION APPROACH**

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**Background**

Our current society is confronted with a series of global geopolitical, economic, energetic and climate crises. While there is no common solution for all these issues, there is an obvious main connection, the need for a novel renewable and environmentally friendly energy carrier. Hydrogen gas is considered an attractive alternative to fossil fuels with certain methods of biohydrogen production being able to utilize various low-priced industrial and agricultural wastes, thus coupling organic waste treatment with renewable energy generation.

**Objectives**

By designing a novel hybrid dark fermentation-photoheterotrophic degradation biohydrogen production process using industrial wastewater as a substrate we aim to resolve the major scientific and technical bottle-necks preventing the biohydrogen production systems to become economically feasible, bringing thus the technology closer to the industry.

**Methods**

A laboratory-scale bioreactor experimental setup was developed in order to identify the most suited microbial and micro-algal consortia, as well as the key metabolic pathways involved. In addition, an advance statistical design of experiments approach was used to identify the influence and interactions of key micro-environmental factors.

**Conclusions**

The obtained preliminary results prove that this novel system has the potential to produce considerably more H<sub>2</sub> yields compared to traditional systems as well as generate valuable oils through the micro-algal metabolic activity. In addition, such a novel technology could capture the generated CO<sub>2</sub> produced during the dark fermentation step, and used it by the micro-algal species together with the dark fermentation effluent.

**FEMS-0990**

**Biotechnology and industrial microbiology**

**EXTRACELLULAR N-ACETYLGLUCOSAMINE INCREASES HYALURONAN YIELD AND ITS MOLECULAR WEIGHT IN CULTURE OF STREPTOCOCCUS ZOOEPIDEMICUS**

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**Background**

Hyaluronan (hyaluronic acid, HA) is a linear polysaccharide composed of alternating D-glucuronic acid (GlcUA) and N-acetylglucosamine (GlcNAc) linked by  $\beta(1,4)$  and  $\beta(1,3)$  glycosidic bonds. This macromolecule is found in various tissues of vertebrates, where it has different biological functions. Industrially, HA has been manufactured by fermentation of group C streptococci.

This polysaccharide is produced by processive synthase from the activated UDP-sugars (UDP-GlcA and UDP-GlcNAc). A positive correlation between molecular weight (MW) and concentration of UDP-GlcNAc was previously reported [1].

**Objectives**

To increase hyaluronan yield or its molecular weight by strengthening UDP-precursors biosynthesis.

**Methods**

Fermentations were carried out in fermentor Sixfors (Infors HT, Switzerland) with working volume of 300 ml. HA yield was determined by isopropanol precipitation. HA Mw was analysed by SEC-MALLS.

**Conclusions**

In order to increase yield or molecular weight of hyaluronic acid in *Streptococcus zooepidemicus* fermentation, cultivation medium was supplemented with GlcUA and GlcNAc. These molecules are involved in hyaluronan biosynthetic pathway.

Extracellular GlcNAc significantly increased both HA yield and molecular weight (35% and 21% respectively). HA yield positively correlated with GlcNAc concentration up to 1g/l. Higher concentrations had detrimental effect on HA production. No significant

effect on HA yield was observed for GlcUA addition.

**FEMS-0993**

**Biotechnology and industrial microbiology**

## **EVALUATION OF BACTERIAL ENZYMES IN DEGRADATION OF TEXTILE DYES**

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### **Background**

Because of the ease of their synthesis and the greatest variety of colors, azo dyes are the largest class of synthetic dyes. Generally they are very recalcitrant to biodegradation. Thus, in the current study the biodegradation of an azo dye namely Remazol Blue (RB) by wastewater- isolated bacteria (*Bacillus megaterium*, *Micrococcus luteus* and *Bacillus pumilus*) has been investigated in media containing molasses as a carbon and energy source.

### **Objectives**

The goal of this work was to identify decolorization and degradation properties of Remazol Blue dye by bacterial strains at batch scale .

### **Methods**

Enzyme activity: The reaction mixture contained 400 µl of 50 mM sodium phosphate buffer (pH 7.0), 200 µl of the sample and 200 µl of Remazol Blue. The reaction was initiated by the addition of 200 µl NADH (final concentration 2 mM) and followed photometrically.

Bioremoval assays: Experiments were carried out at different (from 28.7 to 97.9 mg/L) dye concentrations at pH 7 throughout 3 days.

### **Conclusions**

*Bacillus* -derived azoreductases were responsible for biodegradation of RB. The maximum azoreductase activity was obtained as 39.9 U/ml for *B. pumilus* at 2<sup>nd</sup> day. The removal yields of *B. pumilus* were 71.0%, 74.3%, 67.2% and 69.9% for 28.7, 57.9, 78.3 and 97.9 mg/L dye, respectively. There were also high azoreductase activities for *B. megaterium* at the first and second days of incubation time such as 37.4 and 33.6 U/ml while *M. luteus* did not show any azoreductase activity.

**FEMS-0994**

**Biotechnology and industrial microbiology**

**REMOVAL OF NI(II), CU(II) AND CR(VI) AND EPS PRODUCTION BY BACTERIAL CULTURES ISOLATED FROM ANKARA STREAM**

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**Background**

Among many heavy metals, Cr(VI), Ni(II) and Cu(II) are the ones having many usage fields, and therefore wastewaters including them have to be treated.

**Objectives**

This study aimed to isolate Ni(II), Cu(II), Cr(VI) resistant-bacteria from Ankara Stream (Turkey) and determining EPS production and bioremoval capacities of them under different conditions.

**Methods**

Experiments were done with bacterial cultures in different media (active sludge: AS; active sludge with molasses: MAS) including Ni(II), Cu(II), Cr(VI) at varied pH values (6-9). Heavy metals were determined spectrophotometrically at 340 nm for Ni(II); 460 nm for Cu(II); 540 nm for Cr(VI). EPS amount was also investigated.

**Conclusions**

Mixed bacterial cultures had higher bioremoval than the pure cultures. Six heavy metal-resistant strains were purified. Strain 5 had the highest Cu(II) (69.1%) and Cr(VI) (43.1%) removal under 25 mg/l heavy metal at pH 7. The same strain was the efficient bacterium in bioremoval and EPS production. It had EPS amount as 1.05 g/l at 25 mg/l Cr(VI), and 0.74 g/l at similar Cu(II) concentration. Especially mixed and also pure bacteria could be used as a biosorbent in treatment of heavy metals.



**FEMS-0998**

**Biotechnology and industrial microbiology**

**BIOETHANOL PRODUCTION FROM AGRICULTURAL WASTES BY  
SACCHAROMYCES CEREVISIAE**

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**Background**

The depletion of fossil fuels and the concerns about environment have led scientists to find alternative renewable energy sources. Bioethanol is the most widely used liquid biofuel at present among these alternative fuels. Feedstock costs are the major part of the process. Thus, considerable work has been performed toward production of bioethanol using various kinds of feedstocks such as starch rich agricultural wastes and cellulosic biomass.

**Objectives**

To demonstrate the utilization capacity of agricultural wastes (apple and carrot pomaces) as feedstocks for bioethanol production.

**Methods**

*Feedstock preparation:* Appropriate amounts of apple and carrot pomaces were mixed with distilled water and homogenized to obtain desired concentration of feedstock solutions. Different pretreatment methods were performed to find the most effective way to obtain fermentable sugars in pomaces.

*Fermentation conditions:* The yeast cells were precultured in YPG medium at pH 6. 10% yeast suspension was aseptically transferred to anaerobic fermentation medium which is prepared by pomace sugar containing distilled water.

*Biomass, sugar and ethanol determination:* Yeast biomass was determined by measuring optical density. The sugars were determined by phenol-sulphuric acid method. The bioethanol concentration was analyzed using gas chromatography.

**Conclusions**

Higher ethanol production values were obtained from apple pomace rather than carrot. The pretreatments performing with acid were more effective than the ones

performed with base. The highest ethanol production was obtained when the apple pomace was pretreated with 0.25% (v/v)  $\text{H}_2\text{SO}_4$ .

**FEMS-0999**

**Biotechnology and industrial microbiology**

## **BORON REMOVAL FROM AQUEOUS SOLUTIONS USING RHODOTORULA MUCILAGINOSA AS A BIOSORBENT**

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### **Background**

Boron (B) is a naturally occurring element found in earth's crust, rocks, soil, and water. Although B is an essential element and used at many industries, it could be toxic for organisms in excess concentrations.

### **Objectives**

In the present study we aimed to remove B from aqueous media via biosorption by *Rhodotorula mucilaginosa*, which was isolated from B-contaminated wastewater.

### **Methods**

Batch culture experiments including pH (4-7), increasing B (15-20 mg/L), biomass concentrations (1-4 g/L) and different biosorption methods (wet, dried and formaldehyde-treated cells) were carried out at 25±2 °C, at 100 rpm for 120 min. Boron concentrations were determined spectrophotometrically by measuring the absorbance at 585 nm with using carmine.

### **Conclusions**

According to the data obtained, the optimum pH was found as 6 for the maximum B removal. Under 15.35 mg/L B, the highest biosorption was determined as 23.78%. Biosorption was increased with increasing biomass and the maximum yield (31.92%) was found at 4 g/L biomass. Wet cells removed B more effectively than formaldehyde-treated or dried cells and the yields were 23.78%, 19.31% and 12.07%, respectively. It can be concluded that *R. mucilaginosa* might be an effective biosorbent in B removal processes.

## FEMS-1005

### Biotechnology and industrial microbiology

#### A NOVEL PATHWAY FOR ITACONIC ACID PRODUCTION IN USTILAGO MAYDIS

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#### Background

Itaconic acid is a promising bio-based platform chemical for the production of pharmaceuticals, adhesives and polymers. *Ustilago* is a promising fungal host for the production of itaconic acid. Contrary to established filamentous itaconate producers, *Ustilago* grows unicellularly. This has distinct process advantages relating to morphology control, viscosity and aeration. However, the itaconate production yield, titer and rate of *Ustilago* are below that of the commercial production hosts. In order to optimize itaconate production in *Ustilago*, detailed biochemical knowledge is needed.

#### Objectives

To characterize the itaconate production pathway in *Ustilago* in order to enable metabolic engineering.

#### Methods

An itaconate gene cluster was identified in *Ustilago*. The function of the cluster genes and their encoded proteins was characterized by knockout and overexpression. The activity of two essential catalytic proteins was determined *in vivo* by permeabilized cell assays and *in vitro* by *E. coli* expression and purification.

#### Conclusions

The genes *tad1* (*trans*-aconitate decarboxylase), *itp1* (Major Facilitator Superfamily extracellular itaconate transporter), *adi1* (aconitate- $\Delta$ -isomerase), *mtt1* (mitochondrial tricarboxylate transporter), and *ria1* (transcriptional itaconate regulator) are involved in the itaconate biosynthesis and possibly its further conversion.

In contrast to the known itaconate biosynthesis pathway of *Aspergillus terreus*, *Ustilago*'s itaconate production proceeds first via an isomerization from *cis*- to *trans*-aconitate, followed by decarboxylation of the *trans*-aconitate. First metabolic engineering attempts were successful, enhancing *U. maydis*' itaconate production twofold by overexpression of *ria1* or *mtt1*. This work lays the foundation for further

optimization of *U. maydis*' itaconate biosynthesis and is therefore a further step towards industrial application of this promising biocatalyst.

**FEMS-1006**

**Biotechnology and industrial microbiology**

**PURIFICATION AND CHARACTERIZATION OF A LIPASE WITH HIGH SYNTHETIC ACTIVITY FROM RHIZOPUS STOLONIFER**

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**Background**

Filamentous fungi are extensively studied due to their exoenzyme producing ability. Since lipases are effectively utilizable in various industrial processes, special attention is paid for these enzymes. Development of environmentally friendly technologies requires lipases able to catalyze the rapid synthesis and translocation of esters. Isolation and analysis of such enzymes contributes to the knowledge of these catalytic processes, and provides data on new lipases with potential biotechnological interest.

**Objectives**

A *Rhizopus stolonifer* isolate has been identified as high-yield lipase producer in our previous tests. Further studies revealed that the crude enzyme has significant transesterification activity. Therefore, our present work has focused on the purification and characterization of this *R. stolonifer* lipase.

**Methods**

Isolate was grown on liquid medium containing wheat bran. To purify the enzyme, after filtration and centrifugation, ammonium sulphate precipitation, size-exclusion and ion-exchange separations were performed. Biochemical characterization assays including substrate specificity, temperature and pH tolerance studies, and examination of the effect of some ions and organic solvents on the activity were carried out.

**Conclusions**

The optimal temperature and pH for the activity were about 50 °C and pH 5.0, respectively. The lipase has broad substrate specificity because it has effectively hydrolyzed the substrates containing fatty acids from C6 to C16. Enzyme activity was stimulated by 5% ethanol, butanol and propanol. Results showed that certain alkanes could stabilize the active conformation of the enzyme. The isolated *R. stolonifer* lipase exhibited high esterification and transesterification activity at 40-50 °C. This research was supported by the Hungarian Research Fund (OTKA PD 112234).

**FEMS-1012**

**Biotechnology and industrial microbiology**

**INVESTIGATION OF THE TRANSESTERIFICATION AND ESTERIFICATION  
PROPERTIES OF EXTRACELLULAR LIPASE ENZYMES FROM  
MUCOROMYCOTINA FUNGI**

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**Background**

Recent years, there is a fast-growing interest on the catalytical activities display by lipase enzymes, especially focusing on their synthetic activity in organic solvents. Translocation and synthesis of ester linkages result various aroma-, phenyl- and alkyl esters and different polymers. Many of these compounds have important role in the food-, pharmaceutical-, and oleochemical industries as well as in the biodiesel production. In these industries, development of modern environmentally friendly biotechnological technologies requires the exploration and utilisation of new microbial enzymes.

**Objectives**

Lipases can be produced by certain *Mucoromycotina* fungi as well, but only some of these enzymes have been tested for their synthetic activity in organic media. In this work, we evaluated the esterification and transesterification capacities of *Rhizomucor*, *Rhizopus*, *Mucor* and *Mortierella* lipase enzymes through alkyl ester synthesis, and we characterise of their fatty acid preference during these reactions.

**Methods**

Reactions were carried out in non-aqueous condition using lyophilised crude enzyme preparations. Transesterification was studied between *p*-nitrophenyl-palmitate and ethanol, while esterification reactions were examined with different chain length fatty acids and methanol. The effect of the reaction time on the ester production was also evaluated. Synthetic esters produced were detected by a GC-FID technique.

**Conclusions**

Each lipase catalysed the transesterification significantly faster than esterification. High ethyl-palmitate production was reached by lipases from *Rhizopus stolonifer* and *Mucor corticolus* strains. During esterification reactions, the tested enzymes showed increased alkyl ester synthesis in the presence of medium-chain fatty acids. This

research was supported by the TÁMOP-4.1.1.C-12/1/KONV-2012-0012 and the Hungarian Research Fund (OTKA PD 112234).



## **FEMS-1026**

### **Biotechnology and industrial microbiology**

#### **NOVEL SMALL-SCALE AND ENZYMATIC TEST SYSTEMS TO ANALYSE FULL OPERATING BIOGAS PLANTS**

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#### **Background**

One important renewable energy source is biomass from energy crops and organic waste that is used for the production of biogas. Biogas mainly consists of CH<sub>4</sub> and CO<sub>2</sub> and is generated by anaerobic fermentation of organic matter.

#### **Objectives**

For a more precise and targeted optimization it is crucial to understand the biological processes during biogas production.

#### **Methods**

Using authentic samples from full operating biogas plants it was possible to develop batch and continuous reactors mirroring the conditions and the performance of the full-scale biogas plant. With these test systems it was possible to observe effects of different substrates for syntrophic bacteria and for methanogenic archaea on biogas formation.

Furthermore, the development of novel sensors in biogas plants to quantify the activities of key enzymes is desirable. A potential candidate is the heterodisulfide reductase (Hdr). Methanogenesis finally results in the formation of the heterodisulfide, which is regenerated by the Hdr. In acetoclastic methanogens the Hdr is tightly bound to the cytoplasmic membrane but in hydrogenotrophic methanogens it is located in cytoplasm.

#### **Conclusions**

Most biogas sludge samples were stimulated by the addition of ethanol or acetate, which leads to increasing methane production by 35-126 %. These results indicate that syntrophic ethanol oxidation and acetoclastic methanogenesis were not rate limiting in routinely operating biogas plants.

Our test system allows a quantification of hydrogenotrophic/aceticlastic methanogens in biogas sludge based on Hdr activity. In summary 26% of total Hdr activity was found in the membranes while the cytoplasmic fraction contained 74% of total activity.

**FEMS-1034**

**Biotechnology and industrial microbiology**

**EXPRESSION OF AGARICUS BISPORUS MANGANESE PEROXIDASES IN SCHIZOPHYLLUM COMMUNE**

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**Background**

White rot fungi are thought to degrade lignin using AA2 enzymes like lignin peroxidase, manganese peroxidase (MnP) and versatile peroxidase. It is unclear if *S. commune* is a white rot or brown rot fungus. This basidiomycete is a poor lignin degrader. No genes encoding AA2 enzymes are found in its genome, while it is enriched in AA9 genes. This would place *S. commune* in between brown and white rot fungi. We here introduced two *A. bisporus mnp* genes in *S. commune*.

**Objectives**

Introduction of *A. bisporus* MnPs in *S. commune* and its effect on the lignin degrading capacity of *S. commune*.

**Methods**

qPCR verified expression of these genes in *S. commune*. Decoloration of RBBR by *mnp* expressing *S. commune* strains indicated production of active MnP. This was confirmed using the MBTH/DMAH assay. Addition of hemin to standing cultures increased MnP activity 5-fold. Pyrolysis analysis indicated that a *S. commune* strain expressing both MnPs of *A. bisporus* degraded lignin in birchwood .

**Conclusions**

*A. bisporus* MnPs were successfully introduced and expressed in *S. commune*. The G subunit of lignin seems to be preferentially degraded by *S. commune* MnP expressors. The ligninolytic pathway of litter degrading fungi can be reconstituted in *S. commune*.

**FEMS-1035**

**Biotechnology and industrial microbiology**

**MICHAEL-TYPE ADDITION USING 4-OXALOCROTONAT TAUTOMERASE:  
BIOPROCESS IMPROVEMENT**

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**Background**

Enzyme 4-oxalocrotonate tautomerase (4-OT) encoded by *xylH* gene is a part of aromatic compounds degradation pathway in *Pseudomonas putida* mt-2. Due to its promiscuous activity, 4-OT was described to catalyse Michael-type addition of acetaldehyde to  $\beta$ -nitrostyrene, the isomerisation of *cis*-nitrostyrene to *trans*-nitrostyrene as well as aldol condensation and dehydration as free enzyme. Whole cell system based on recombinantly expressed 4-OT has been developed and shown to be effective biocatalyst of asymmetric Michael addition of acetaldehyde to  $\beta$ -nitrostyrene.

**Objectives**

The objective of this study was to improve biocatalytic production of 4-nitro-3-phenylbutanal employing two strategies: biocatalyst immobilization and product recovery using Amberlite XAD-2 polymeric adsorbent.

**Methods**

Whole cell biocatalyst was immobilized in 4% alginate gel. Capsules of 1 and 2 mm in diameter were made. Liquefaction of the capsules core was performed by addition of sodium citrate. Depletion of  $\beta$ -nitrostyrene was monitored by reduction of absorbance at 320 nm. Product was recovered from aqueous reaction buffer by addition of XAD-2 beads, followed by elution in minimal volume of ethylacetate.

**Conclusions**

Biocatalytic production of 4-nitro-3-phenylbutanal was improved by whole-cell immobilization in alginate capsules. Capsules were used in 12 reaction cycles, and retained 20% of the activity after two months of storage. Bioprocess was further improved by using XAD-2 beads for improved product recovery. With this

modification, the amount of organic solvent was reduced 20-fold in comparison to previously reported method making this process more environmentally friendly.

**FEMS-1042**

**Biotechnology and industrial microbiology**

**COMPARATIVE GENOMIC ANALYSIS OF SOLVENT PRODUCING  
CLOSTRIDIUM ACETOBUTYLICUM AND CLOSTRIDIUM BEIJERINCKII STRAINS**

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**Background**

The clostridial acetone-butanol-ethanol (ABE) fermentation pathway is complex and represents one of the oldest industrial fermentations. Interest in the production of solvents from lignocellulosic sources has increased in recent years. However, low production yields, end-product inhibition and phage infections remain as disadvantages in the ABE fermentation.

**Objectives**

Genetic and metabolic engineering strategies leading to a stable, high-yielding process require prior genomic exploration of diverse solventogenic Clostridial strains.

**Methods**

Ten strains of *C. acetobutylicum* and *C. beijerinckii* were selected from batch fermentation experiments according to their solvent yields. Genomes and plasmids (when present) were sequenced using the Illumina HiSeq platform. Complete genomes were obtained through *de novo* and reference-guided assembly comparison with coverages of ~ 100x. Annotation was performed using RAST (<http://rast.nmpdr.org>). Key enzymes for solvent production were mapped in the genomes and/or plasmids to allow variant detection and comparison in future RNA-seq experiments. Raw Illumina reads were used as input for CRISPR structure detection using the Crass software (<http://ctskennerton.github.io/crass/>). Putative CRISPR fragments were assembled and queried against the CRISPR database (<http://crispr.u-psud.fr/crispr/>). Complete CRISPR structures were detected in 7 out of 10 genomes (average 2.5 CRISPR structures per strain), and repeat/spacer units were detected in all 10 sequenced strains. An alignment-based approach revealed the existence of uneven distribution of types (11 detected) across their genomes.

**Conclusions**

In combination, our results provide a sound basis for directed generation of engineered Clostridial solventogenic strains, with the potential to re-launch the ABE fermentation as a competitive industrial fermentation.



**FEMS-1056**

**Biotechnology and industrial microbiology**

## **REROUTING OF CARBON FLUX TOWARDS GLYCEROL PRODUCTION IN YEAST *SACCHAROMYCES CEREVISIAE***

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### **Background**

Currently there is an interest in development of microbial strains effectively converting cheap feedstocks to glycerol. Facultative anaerobic yeast *Saccharomyces cerevisiae* can be a good platform for development of recombinant strains overproducing glycerol under low-aeration conditions. In *S. cerevisiae* glycerol is synthesized from dihydroxyacetone phosphate by the action of glycerol-3-phosphate dehydrogenase (Gpd1) and glycerol-3-phosphate phosphatase (Gpp2). Other road of dihydroxyacetone phosphate catabolism occurs by its isomerization to glyceraldehyde-3-phosphate with triose phosphate isomerase (Tpi1), resulting to ethanol formation.

### **Objectives**

We aimed to construct recombinant strains with reduced Tpi1 activity and increased Gpd1 and Gpp2 activities.

### **Methods**

Homologous recombination was used for partial substitution of *TPI1* gene promoter region with selective marker. Obtained strains contain 100, 50 or 25 base pairs of native *TPI1* gene promoter before *TPI1* ORF, and revealed corresponding sequential decreases in Tpi1 activity. Multicopy integration module was used for expression of hybrid *GPD1-GPP2* ORF (encoding artificial fusion of both enzymes) under the control of strong constitutive promoter of the alcohol dehydrogenase gene.

### **Conclusions**

Recombinant *S. cerevisiae* strains with 50 or 25 bp version of *TPI1* promoter revealed up to 2 times increase in glycerol production as compared to the WT strain. Recombinant strains expressing *GPD1-GPP2* fusion produced approximately 4 times more glycerol than that of WT strain. Combination of both approaches resulted to 5-fold increase of glycerol production as compared to the WT strain.



**FEMS-1068**

**Biotechnology and industrial microbiology**

**METHANOTROPHIC EXTREMOPHILES: FROM UNUSUAL PHYSIOLOGY TO NEXT GENERATION BIOCATALYSIS**

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**Background**

The long-term goal of this research is to develop a robust microbial platform for conversion of greenhouse gases (CH<sub>4</sub> and CO<sub>2</sub>) into valuable chemicals as an alternate approach to widespread (bio)gas-flaring.

**Objectives**

The main objective of this project is to generate a comprehensive vision of methane oxidation in a model methanotroph (*Methylobacterium alcaliphilum* 20Z) using modern systems biology approaches.

**Methods**

A multi-tiered systems-level approaches (genomics/mutagenesis, transcriptomics, metabolomics and fluxomics) were used to validate the metabolic network in *M. alcaliphilum* 20Z grown on methane and methanol during both, steady state and transitions from restricted to active utilization of C1-substrate.

**Conclusions**

Comparisons of different growth conditions highlighted functions important for methane oxidation and for the re-activation of methane oxidation machinery. Complete genome sequences of the methanotrophic strain enabled genome-wide metabolic reconstruction. The first stoichiometric flux balance model of methane utilization was constructed and validated. A new vision of the core methanotrophic functions will be presented. The majority of the data (methane oxidation parameters, growth yield, mutant phenotypes) suggest that *direct coupling* between methanol and methane oxidation is the most compelling mode of methane activation. The reconstruction of spatial organization of the metabolic network coupled with the computational modeling enables a mechanistic understanding and systematic analysis of the interplay between core methane oxidation processes and overall performance of the biological system. New data were used to improve conversion of the C1-substrate into value-added chemicals and fuels.



**FEMS-1074**

**Biotechnology and industrial microbiology**

**OPTIMIZATION OF LEVAN PRODUCTION BY BACILLUS LICHENIFORMIS  
ANALYSED BY THE RESPONSE SURFACE METHOD**

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**Background**

Levan is a fructose based exopolysaccharide produced by microorganisms as an energy reserve and defense. It has present low viscosity, high solubility in water, biocompatibility and other properties that have industrial and medicine applications. Levan-type polymers have been reported as hypocholesterolemic, cell-proliferating, anti-inflammatory and antitumour agent, an immune modulator, thickener, stabilizer, flavors, and a aroma carrier [1].

Response surface methodology (RSM) is the most popular optimization method used in recent years because the application of statistical designs for experiments and its modeling defines the effect of various factors and its interaction [2].

**Objectives**

The aim of this study was to optimize fermentation medium composition for levan production by *Bacillus licheniformis* strain.

**Methods**

Basic medium for levan production contains sucrose as carbon source, yeast and beef extract as nitrogen source, and its pH level is important for excretion of polysaccharide. In this work it has been shown that high levan production by the studied strain could be achieved with ammonium chloride as a sole nitrogen source. Influence of three independent variables (pH, sucrose and ammonium-chloride concentration) was investigated using Box-Behnken statistic design.

**Conclusions**

Based on response surface model that we obtained, in medium that contain 200 g/L sucrose, 1.44 g/L ammonium-chloride and pH 7.5 maximum concentration of levan (41.29 g/L) is reached, which was in agreement with experimental data.

**References:**

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**FEMS-1106**

**Biotechnology and industrial microbiology**

**MOLECULAR BREEDING OF YEAST CO-DISPLAYING ENDO- AND EXO-TYPE ALGINATE LYASES TO COMPLETELY UTILIZE MACROALGAE**

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**Background**

Brown macroalgae is characterized by a large size and high productivity without requiring arable land, irrigation water, or fertilizer. It is a raw material suitable for productivity of bioenergy and biochemical productions. However, alginate, which is the main component of brown macroalgae, is an undegradable polysaccharide with poly (M)-, poly (G)-, and poly (MG)-blocks composed of  $\alpha$ -l-guluronic acid (G) and its C5 epimer,  $\beta$ -d-mannuronic acid (M), as monomeric units.

**Objectives**

In order to degrade alginate polymers effectively, both endo- and exo-type alginate lyases are necessary. Therefore, we co-displayed endo- and exo-type alginate lyases from the alginate-degrading marine bacterium *Saccharophagus degradans* [1] on the cell surface of *Saccharomyces cerevisiae* with our original method, cell surface engineering technology [2].

**Methods**

The alginate lyases from *S. degradans* were produced as fusion proteins with cell wall anchoring domain of  $\alpha$ -agglutinin in *S. cerevisiae*. The production and localization of alginate lyases on the yeast cell surface were confirmed by immunofluorescence microscopy. The enzyme activities of the displayed alginate lyases were measured by using the dinitrosalicylic acid method.

**Conclusions**

The results suggested that the engineered yeasts co-displaying endo- and exo-type alginate lyases could lead to efficient production of monomeric units from alginate polymers [3]. This system is a unique easily feasible to utilize any biomass.

**References**

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**FEMS-1134**

**Biotechnology and industrial microbiology**

**SCREENING AND MOLECULAR CHARACTERIZATION OF FUNGI CAPABLE OF LACCASE PRODUCTION FROM DIFFERENT FOREST ECOSYSTEMS OF MICHOACÁN STATE, MEXICO.**

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**Background**

Contrary to temperate regions, knowledge on laccase producing fungal species from ecosystems allocated at neotropical regions is still scarce. Search for ligninolytic fungi in these ecosystems can provide laccases with novel biotechnological potential.

**Objectives**

The goal of this study was to explore novel fungal species with laccase activity from different forest ecosystems.

**Methods**

Fungal species were isolated from soil and basidiocarp samples collected from five different geographic regions with dominant vegetation of *Pinus* spp., *Abies* spp., and *Quercus* spp. Extracellular laccase activity by fungal isolates were analyzed by guaiacol assay. Identification of laccase positive fungi was carried out by sequence comparison and phylogenetic analysis of internal transcribed region (ITS1F-5.8S-ITS4B) of rDNA gene with reference taxa.

**Conclusions**

A total of thirty (30) laccase producing fungi, representing twenty one (21) species of fifteen (15) genera, were purified. Fifteen strains (15) with high laccase activity of basidiomycetes were identified in thirteen genera i.e., *Bjerkandera*, *Corticium*, *Coriopsis*, *Echinodontium*, *Ganoderma*, *Hexagonia*, *Irpex*, *Limonomyces*, *Psathyrella*, *Peniophora*, *Phlebiopsis*, *Trametes* and *Trichaptum*. Seven (7) *Trichoderma* and only one *Penicillium* species were found as low laccase producer, isolated from different soil and basidiocarp samples. *Trichoderma tomentosum* was recorded as most isolated laccase producing isolate from different soil samples

followed by *T. atroviride* from different regions. However, *Penicillium pinophilum* was only laccase producing species of genus *Penicillium*, isolated from soil sample of *Pinus* spp., and *Quercus* spp., dominant area.



**FEMS-1189**

**Biotechnology and industrial microbiology**

**COMPLEX PROBIOTIC FEED SUPPLEMENT BASED ON CONSORTIUM OF MICROBIAL STRAINS FOR INDUSTRIAL POULTRY BREEDING**

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**Background**

Large-scale poultry breeding often provokes outbreaks of various bacterial, viral and mixed infections leading to fowl loss, while shortage of vitamins and hardly digestible feed ingredients reduces nutritive value and downgrades commodity outlook for meat and egg products.

**Objectives**

Aim of this study – development of technology for production and application of complex probiotic feed additive possessing antimicrobial, antiviral and antioxidant action allowing to prevent and cure fowl diseases, to raise nutritive value and digestibility of fodder, to promote poultry resistance to stress factors.

**Methods**

Microbiological, biochemical, genetic engineering, physical-chemical methods were used in this research: engineering a strain-producer of chicken leukocyte alpha-interferon, submerged fermentation of microorganisms, Ispectrometry, freeze-drying and etc.

**Conclusions**

To broaden the activity spectrum of probiotic feed supplement several bacterial species capable to complement each other were applied. Active principle of the feed additive — strain *Bacillus subtilis* produces surfactins, polyene and peptide compounds displaying antagonistic activity against strictly and facultatively pathogenic microbial species colonizing avian gastrointestinal tract. It was found that *Bacillus subtilis* bacteria generate cellulases, proteases and other enzymes facilitating assimilation of fodder. Enterobacteria *Pantoea agglomerans* synthesize carotenoid pigments – sources of antioxidant activity and precursors of vitamin A in the body. The protein of chicken leukocytic alpha-interferon synthesized by *Escherichia coli* strain possesses pronounced non-specific antiviral and immune-stimulating activities.

Technology of producing complex probiotic feed supplement in dry form for poultry was developed.

Biological trials *in vitro* and *in vivo* demonstrated its safety and high efficiency in feeding experiments with broilers and hens.



**FEMS-1191**

**Biotechnology and industrial microbiology**

**EXPLORING THE COMPLEX MICROBIAL COMMUNITY OF ACTIVATED SLUDGE SAMPLED FROM AN INDUSTRIAL TANNERY WASTEWATER TREATMENT PLANT BY ILLUMINA MISEQ.**

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**Background**

Background: In the frame of the BIOSUR project (LIFE11 ENV/IT/075), a one-year monitoring of an industrial tannery wastewater treatment plant was performed, both from chemical-physical and biological points of view.

**Objectives**

Objectives: This study is conducted to investigate the evolution of the bacterial community of activated sludge in the same treatment plant during 2013.

**Methods**

Methods: Bacterial diversity is analyzed by the use of the Illumina MiSeq platform for sequencing hypervariable regions V3-V4 in the 16S rRNA genes. Bioinformatic analysis of the obtained 12,429,502 paired-ends reads is performed using the QIIME pipeline. The assembled 4,039,284 contigs are binned into 5,156 Operational Taxonomic Units (OTUs) that are assigned to taxonomic groups by the use of the Silva 111 database.

**Conclusions**

Conclusions: Preliminary results indicate that the bacterial diversity of active sludge is maintained at very high levels throughout the whole year. The dominant bacterial *phylum* is *Proteobacteria*, which accounts for 40%. Multivariate analysis shows that there is a discontinuity between two groups of samples. The first group, corresponding to samples collected from January to July 2013, is detached from the second group, corresponding to samples collected from September, after a period of drastic decrease of work in tanneries. The Shannon diversity index (*H*), used to calculate the diversity of bacterial communities, indicates a slight increase in the diversity level in the second period. Further analysis will better clarify the complexity

of the microbial community and the influence of specific environmental parameters on its structure and evolution.

**FEMS-1196**

**Biotechnology and industrial microbiology**

**IMPROVEMENT OF GLYCEROL PRODUCTION BY DELETION OF ADH1 GENE AND OVEREXPRESSION OF GPD1 AND GPP2 GENES IN SACCHAROMYCES CEREVISIAE**

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**Background**

Glycerol is widely used in cosmetics, food, tobacco, pharmaceutical, leather and textile industries. In addition, it is considered as a cheap raw material for microbial fermentation. That is why the construction of yeast strains-producers of glycerol became an actual objective for modern metabolic engineering. Our strategy comprised the deletion of *ADH1* gene, encoding alcohol dehydrogenase in *S. cerevisiae* and overexpression of both glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase genes (*GPD1*, *GPP2*) in order to redirect the carbon flux to glycerol instead of ethanol formation.

**Objectives**

Our goal was to design recombinant strains with deletion of *ADH1* gene combined with increased Gpd1 and Gpp2 activities.

**Methods**

Homologous recombination was used for deletion of *ADH1* gene with selective marker. The integrative module was used for expression of hybrid *GPD1-GPP2* ORF (encoding artificial fusion of both enzymes) under the control of strong constitutive promoter of the alcohol dehydrogenase gene.

**Conclusions**

Glycerol concentration during the course of fermentation was approximately 4.7-fold enhanced in a  $\Delta adh1\_GPD1-GPP2$  recombinant strain if to compare with BY4742 (2.57 g/L), reaching 12.14 g/L (0.168 g/L/h) on the third day of fermentation. Furthermore, the glycerol production in a recombinant  $\Delta adh1\_GPD1-GPP2$  strains with deletion of *ADH1* gene and overexpression of *GPD1* and *GPD2* genes was nearly 1.2-fold increase in comparison with  $\Delta adh1$  strain, which contained only the disruption of *ADH1* gene.



**FEMS-1200**

**Biotechnology and industrial microbiology**

**MODIFICATION OF METHABOLIC PATHWAY FOR GLYCEROL PRODUCTION IN YEAST *SACCHAROMYCES CEREVISIAE***

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**Background**

Several processes are known for the microbial synthesis of glycerol from carbohydrates that use osmotolerant yeasts, algae and a number of bacteria. However, all these utilize aerobic conditions, so the glycerol production demands air or oxygen purging throughout the fermentation, which considerably increases production costs. Therefore the development of the *Saccharomyces cerevisiae* strains capable of efficient glycerol production from glucose under anaerobic conditions is of great interest.

**Objectives**

We aimed to increase glycerol production by derepression of *ILV2* in part with *GPD1-GPP2* fused genes.

**Methods**

Homologous recombination was used for construction of the truncated version (deficient 5'-165 bp and lacking a mitochondrial targeting signal) of the yeast *ILV2* gene, encoding for acetolactate synthase with a strong constitutive promoter. Obtained strains shown increased acetolactate synthase activity. Multicopy integration module was used for expression of hybrid *GPD1-GPP2* ORF (encoding artificial fusion of glycerol-3-phosphate dehydrogenase and glycerol-3-phosphate phosphatase) under the control of strong constitutive promoter of the alcohol dehydrogenase gene.

**Conclusions**

Overexpression of the truncated version of *ILV2* with presumably cytosolic localization, strongly activates glycerol production under anaerobic conditions. Preliminary results possessed that the glycerol production by recombinant strains co-expressing *ILV2* and *GPD1-GPP2* was increased as compared to the wild type strain and strain expressing solely *ILV2*.

**FEMS-1206**

**Biotechnology and industrial microbiology**

**AN ANTIFUNGAL PEPTIDE FROM ACTINOBACTERIA (STREPTOMYCES SP. TKJ2) : ISOLATION AND PARTIAL CHARACTERIZATION**

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**Background**

Actinobacteria are of special biotechnological interest since they are known to produce chemically diverse compounds with a wide range of biological activity. This distinct clade of Gram-positive bacteria include some of the key antibiotic producers and are also sources of several bioactive compounds

**Objectives**

the aim of our study is the purification and the partial characterization of an antifungal protein produced by *Streptomyces* sp. TKJ2 isolated from Algerian soil.

**Methods**

A newly filamentous bacteria was recovered from Tikjda forest soil (Algeria) for its high antifungal activity against various pathogenic and phytopathogenic fungi. The nucleotide sequence of the 16S rRNA gene (1454 pb) of *Streptomyces* sp. TKJ2 exhibited close similarity (99 %) with other *Streptomyces* 16S rRNA genes. Antifungal metabolite production of *Streptomyces* sp TKJ2 was evaluated using six different fermentation media. The antifungal protein produced by *Streptomyces* sp. TKJ2 on PCA medium has been purified by ammonium sulfate precipitation, SPE column chromatography and high-performance liquid chromatography in a reverse-phase column.

**Conclusions**

The UV chromatograms of the active fractions obtained at 214 nm by NanoLC-ESI-MS/MS have different molecular weights. The F20 Peptidique fraction obtained from culture filtrat of *Streptomyces* sp. TKJ2 precipitated at 30% of ammonium sulfate was selected for analysis by infusion ESI-MS which yielded a singly charged ion mass of 437.17 Da.



**FEMS-1222**

**Biotechnology and industrial microbiology**

**CONSORTIUM OF ANTAGONISTIC MICROBIAL SPECIES WITH HYDROLYTIC POTENTIAL AS A BASIS OF BIOPREPARATION TO PROMOTE RECOVERY OF SOIL MICROFLORA AND INCREASE CROP PRODUCTIVITY**

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**Background**

In recent years public emphasis has been focused on the problems of rapid arable land degradation: structural deterioration of ploughed layer, reduced humus content, rising acidity – all leading to loss of soil suppressive potential. Tillage quality downward trend requires urgent measures for preservation and remediation of agricultural resources. A vital method to promote soil fertility and harvests of cultivars envisages ploughing-in of straw from crop rotation precursor treated by phytoprotective microbial preparation accelerating decomposition of plant residues and regeneration of soil biocenoses.

**Objectives**

Aim of this study was to assemble a consortium of microbial species distinguished by phytoprotective and hydrolytic potential.

**Methods**

Various investigation methods were used: microbiological, biochemical, molecular-genetical.

**Conclusions**

500 microorganisms isolated from diverse natural sources were screened to yield 33 antagonists of crop soil pathogens. 3 antagonistic bacterial strains were found to show the highest levels of antimicrobial and cellulolytic activities. The selected variants were characterized by the following properties:

- growth suppression of phytopathogens belonging to genera *Fusarium*, *Botrytis*, *Colletotrichum*, *Chaetomium*, *Pseudomonas*, *Xanthomonas*;
- increased seedling/root size and crude weight;

- production of hydrolytic enzymes – endo-1,4- $\beta$ -glucanase and xylanase;
- 10-12% reduction of straw fiber content by 1 month;
- upgrading soil cellulolytic activity;
- stimulating effect on major ecologo-trophical microbial groups in soil (ammonifying, oligonitrophilic, cellulolytic, micromycetes).

The obtained data evidence attractive application prospects of microbial consortium as active ingredient of biopreparation to trying down infectious background, to reconstitute soil microbial cenoses and to promote plant growth.

**FEMS-1224**

**Biotechnology and industrial microbiology**

**SULFUR OXIDIZING BACTERIA COMMUNITY: CHARACTERIZATION AND MONITORING IN A REACTOR FOR SULFIDE REMOVAL FROM INDUSTRIAL WASTE.**

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**Background**

Tannery wastewater contains high concentrations of pollutants, mostly sulfides and sulfates, which are usually chemically removed by expensive and inefficient methods. An innovative oxidizing bioreactor for biological removal of sulfide from gaseous industrial waste streams is developed and set up in the frame of the BIOSUR project (LIFE11 ENV/IT/075).

**Objectives**

This is achieved thanks to the development of a specialized chemolithoautotrophic sulfur-oxidizer biomass, which has been studied in order to elucidate its evolution.

**Methods**

The involved microbial community is selected in a side reactor by incremental addition of sulfides in selective conditions: low pH (2-4) and a concentration of sulfides of 2-4 mg/l. Such a specialized biomass was characterized and monitored by means of T-RFLP fingerprinting, clone-library construction of 16S rRNA coding genes and isolation in pure culture.

**Conclusions**

Preliminary results show the evolution of a selected, specialized sulfur-oxidizer biomass from the onset of the reactor to the stationary phase. In this scenario, about 65% of screened clones are represented by bacteria whose 16S rRNA gene sequence shows a similarity higher than 97% with that of members of the genus *Halothiobacillus*, well-known as Sulfur Oxidizing Bacteria (SOB). The same results has been also confirmed by isolation in SOB selective medium. Finally, obtained data have been comparatively analyzed in order to monitor microbial community evolution from the start-up throughout the end of the experiment.



**FEMS-1236**

**Biotechnology and industrial microbiology**

## **IMPROVEMENT OF GLUTATHIONE PRODUCTION IN METHYLOTROPHIC YEAST *HANSENULA POLYMORPHA***

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### **Background**

Glutathione ( $\gamma$ -L-glutamyl-L-cysteinyl-glycine; GSH) is the most abundant non-protein thiol compound of the most living organisms that protects cells from environmental and oxidative stresses. This tripeptide is an important natural compound for medicine, biotechnology and food industry. Microbial GSH overproduction is limited by mechanisms of feedback inhibition of  $\gamma$ -glutamylcysteine synthetase (GCS), the first and rate-limiting enzyme of GSH biosynthesis, by the end product. In addition the expression of gene coding for GCS is repressed by GSH. Thermotolerant methylotrophic yeast *H. polymorpha* is considered as promising organism for genetic modification and design of competitive GSH producer.

### **Objectives**

To obtain a competitive producer of GSH in methylotrophic yeast *H. polymorpha* by overexpression of modified GCS with eliminated repression-inhibition mechanisms normally exerted by GSH.

### **Methods**

Standard methods of yeast molecular genetics, yeast biochemistry and physiology have been used. The modified versions of *GSH2* gene were obtained by error prone PCR.

### **Conclusions**

The modified versions of *GSH2* gene obtained by error prone PCR were cloned under the control of strong constitutive promoter of glyceraldehyde-3-phosphate dehydrogenase in replicative plasmid pYT3. Selected transformants were analyzed for their resistance to different prooxidant agents (1,2,3-triazole, diethylmaleate, ethionine) as compared to strains carrying unmodified *GSH2* gene. Selected strains possessed growth on medium supplemented with triazole. Strains providing more intensive growth on the selective medium revealed higher GSH accumulation as compared to strains carrying unmodified *GSH2* gene, indicating the reduction of Gsh2 feedback inhibition. In this work the selection scheme providing generation of

Gsh2 insensitive to feedback inhibition was developed.

**FEMS-1247**

**Biotechnology and industrial microbiology**

**HOW TO INTERPRET GENOMIC UNITS INSTEAD OF COLONY FORMING UNITS:  
A CASE STUDY WITH LEGIONELLA PNEUMOPHILA**

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**Background**

*Legionella pneumophila* has been proven to be the causative agent of the potential deadly Legionnaires' disease since 1977. In most of the European countries, the reference method for enumeration of *Legionella* in water is culture (ISO 11731). Due to the long incubation time of this method, more and more laboratories use quantitative PCR (qPCR). Since 2012 the normalization for the *Legionella* qPCR increases considerably: the ISO 12869 was developed, there exist different interlaboratory tests and reference standards. Different reports showed that this technique is accurate enough to be implemented as a reference method. One remaining obstacle is the data interpretation: the culture is expressed in colony forming units (CFU) and the qPCR in genomic units (GU). Although there is a correlation between both units, there is no consensus on how GU can be translated to CFU.

**Objectives**

Based on a database of hot and cold sanitary water samples and a literature screening an action level was defined for the monitoring of *Legionella* in sanitary water.

**Methods**

200 water samples were analyzed by culture and qPCR following the ISO 11731 and ISO 12869 respectively. A correlation analysis was performed and the variance was calculated by the logarithmic differences between the culture and the qPCR results. Together with the results from literature, action levels for the *Legionella* monitoring are proposed.

**Conclusions**

Although the culture has been widely used for many years, one should consider the advantages of qPCR over culture and acknowledge its suitability as a reference method.

**FEMS-1250**

**Biotechnology and industrial microbiology**

**A NOVEL BETA-GALACTOSIDASE FROM ARTHROBACTER SULFONIVORANS:  
PURIFICATION, CHARACTERIZATION AND GENE CLONING**

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**Background**

**Beta-galactosidase** (EC 3.2.1.23) also known as lactase is the enzyme that typically catalyzes hydrolysis of beta-1,4-D-galactosidic linkages in beta-D-galactosides, including disaccharide lactose, with glucose and galactose as end reaction products. This enzyme is able to catalyze synthesis of oligosaccharides, in particular galactooligosaccharides via galactosyl transfer reaction.

*Arthrobacter sulfonivorans* beta-galactosidase of unique for prokaryotes extracellular localization may find application in food industry for manufacturing lactose-free dairy products and in pharmacology as bioactive principle of medicines prescribed for patients suffering from lactase deficiency.

**Objectives**

The study was aimed at cloning of the gene encoding *A. sulfonivorans* beta-galactosidase, purification and characterization of the enzyme.

**Methods**

Fast protein liquid chromatography, electrophoretic analysis, enzyme assay, PCR, DNA sequencing.

**Conclusions**

A novel extracellular beta-galactosidase from *A. sulfonivorans* was recovered with an overall 207-fold purification, a 7.7% yield and specific activity 16 300 U·mg<sup>-1</sup> protein. The subunit molecular mass of the enzyme determined by SDS-PAGE analysis equalled 125 kDa. It was found that the enzyme displays pI 5.35, prefers ortho-nitrophenyl-beta-galactoside as substrate (K<sub>m</sub> 27 mM) and shows maximum activity at 40°C and at pH 7.5–9.5.

The beta-galactosidase gene was isolated from the genomic DNA library of *A. sulfonivorans*, sequenced, cloned and deposited in the GenBank database under



accession number KM277894.1. It was established that the gene carries an open reading frame consisting of 3132 bp (1043 amino acids) and encodes beta-galactosidase referred to Glycosyl Hydrolase Family 2 (CAZy database).

**FEMS-1262**

**Biotechnology and industrial microbiology**

**FUNCTIONALITY ANALYSIS OF STRUCTURAL DOMAINS FROM GD-95 LIPASE BY SITE SPECIFIC AND RANDOM MUTAGENESIS**

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**Background**

The rapid evolution of bioconversion and ecotechnology strongly increases the focus on the enzymes which possess novel properties. One of the most interesting enzymes are lipases from *Geobacillus* bacteria, because they can be active at extreme conditions. The ability to manipulate these lipases depends on the knowledge about the importance on the functionality of their individual domains and amino acids.

**Objectives**

Determination of influence conservative amino acids located at C-terminal end on activity GD-95 lipase without 10 C-terminal amino acids (GD-95-10) and construction new GD-95 lipase variants using error-prone PCR.

**Methods**

Lipase from *Geobacillus* sp. 95 without 10 C-terminal acid was analyzed by Ala scanning mutagenesis. New variants of recombinant GD-95 lipase were created using several error-prone PCR (epPCR). Ala mutants and GD-95 lipase variants after epPCR were cloned into pTZ57R/T and pET-21c(+) vectors. The recombinant proteins were expressed in *Escherichia coli* BL21(DE3) and purified using affinity chromatography. The activity and thermostability analysis of new lipases was performed spectrophotometrically.

**Conclusions**

GD-95-10 lipase was analyzed in this work using Ala mutagenesis. New results confirmed, that higher influence on activity and functionality of GD-95-10 lipase make Asp371 and Tyr376. Also the new GD-95 lipase variants were created using epPCR. The analysis of lipases obtained after random mutagenesis was showed that only a few changes in amino acids sequence can result new physicochemical properties of *Geobacillus* lipases. Therefore our results are basis for further enzyme engineering experiments and for creation of new lipases for bioconversion and ecotechnology.



**FEMS-1276**

**Biotechnology and industrial microbiology**

**USE OF SIGMA FACTORS FROM BACILLUS SUBTILIS IN THE DEVELOPMENT OF AN ORTHOGONAL EXPRESSION SYSTEM IN ESCHERICHIA COLI.**

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**Background**

Technological advances in synthetic biology, systems biology, and metabolic engineering have boosted applications of industrial biotechnology for an increasing number of complex and high added-value molecules. In general, the transfer of multi-gene or poorly understood heterologous pathways into the production host leads to imbalances due to lack of adequate regulatory mechanisms. Hence, fine-tuning expression of synthesis pathways in specific conditions is mandatory.

**Objectives**

Here we develop a new genetic circuit for regulated expression specifically in stationary phase due to clear advantages during this period (reduction of toxicity, competition).

**Methods**

This circuit consists of a heterologous sigma factor ( $\sigma$ ) recognizing specific promoter sequences, which are not recognised by the native  $\sigma$  factors of *E. coli* and is expressed upon entering the stationary phase. First, several  $\sigma$  factors of *B. subtilis* were tested for their orthogonality in *E. coli* on the level of promoter recognition, by using a red-fluorescent reporter system. Secondly, the potential of  $\sigma$  factors of *B. subtilis* to work together with the *E. coli* core RNA polymerase was tested, by expressing these proteins together with their promoters. Based on the results a specific factor will be chosen for further optimisation and the corresponding gene can be cloned in the  $\sigma_S$  factor operon of *E. coli*, which is most abundantly expressed in stationary conditions.

**Conclusions**

Combining all these elements should allow us to create an orthogonal genetic circuit that is able to transcribe specific genes under stationary phase with a limited influence on the host cell's metabolism.

**FEMS-1285**

**Biotechnology and industrial microbiology**

## **COMPARISON OF POLYETHYLENE TEREPHTHALATE DEGRADING POLYESTER HYDROLASES FROM ACTINOMYCETES**

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### **Background**

Polyethylene terephthalate (PET) is a synthetic aromatic polyester that is degraded by several hydrolases from actinomycetes. The heterogeneous enzymatic PET hydrolysis occurs at mild temperature and pH conditions and offers an environmentally friendly alternative to chemical plastic recycling processes. LC cutinase, TfCut2 and Cut190 are homologous polyester hydrolases produced by actinomycetes obtained from a compost metagenome, from *Thermobifida fusca* KW3 and *Saccharomonospora viridis* AHK 190, respectively.

### **Objectives**

The enzymatic degradation of PET films by the three polyester hydrolases was compared at various reaction conditions to evaluate the effects of buffer composition, pH, ionic strength and metal ions on their enzymatic activity.

### **Methods**

Genes coding for LC cutinase, TfCut2 and Cut190 were cloned into pET-20b(+) vector for heterologous protein expression in *Escherichia coli* BL21(DE3). Purified enzymes were used to degrade PET films at 60 to 70 °C for 1 to 50 h in different buffer systems as well as in the presence of Ca<sup>2+</sup> and Mg<sup>2+</sup>. The released products were analyzed by reversed phase high performance liquid chromatography. The weight losses of PET films following an enzymatic hydrolysis by the different enzymes were determined gravimetrically.

### **Conclusions**

The LC cutinase from the compost metagenome and TfCut2 from *T. fusca* showed significantly higher PET hydrolytic activity compared to Cut190 from *S. viridis*. The addition of both Ca<sup>2+</sup> and Mg<sup>2+</sup> stabilized the enzymes and thus facilitated an effective PET degradation. The enzymatic activity of the polyester hydrolases against PET films was strongly depending on the type and ionic strength of the selected buffer.

**FEMS-1296**

**Biotechnology and industrial microbiology**

## **A DISRUPTIVE INNOVATION FOR AMBIENT TEMPERATURE PRESERVATION OF MICROORGANISMS**

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### **Background**

Because of the increase in the number of samples of microorganisms to be stored and shipped, classical preservation in freezers, subjected to risks of technical failure, is becoming more and more costly in space, energy and maintenance. Freeze-drying offers an alternative to cold preservation. However, it is time-consuming and requires precise cycle adjustments. Moreover, glass vials are subjected to breakage and can become leaky resulting in decrease in bacterial viability over time. Imagene innovation aims at preserving dehydrated bacteria at ambient temperature (AT) in hermetic minicapsules that maintain an anoxic and a low-controlled humidity environment. The minicapsules are resistant to impacts and have a unique, engraved datamatrix code allowing a tamper-proof traceability of the samples.

### **Objectives**

This study aims at demonstrating the effectiveness of a novel and rapid process for a reliable long term AT storage of microbial strains.

### **Methods**

Two bacteria, *Pantoea dispersa* and *Aeromonas Salmonicida*, a freeze-drying sensitive strain, were desiccated by evapo-concentration in presence of different stabilization solutions and encapsulated in minicapsules, which were stored either at AT or 37 °C (long-term storage simulation). Bacteria were recovered by simple rehydration.

### **Conclusions**

The residual viability rates post-dessication were ~80 % for *P. dispersa* and ~60 % for *A. salmonicida*. Both strains kept a residual viability rate >50 % after 4 weeks at 37 °C and at AT in Imagene minicapsules. Viability rates following Imagene process were comparable to that of freeze-drying. In conclusion, our work demonstrated that this new process is suited to AT preservation of bacteria.

**FEMS-1297**

**Biotechnology and industrial microbiology**

## **THE HIGH EFFICIENCY FUNGUS (ANTRODIA CINNAMOMEA) FERMENTER FOR TRITERPENOID PRODUCTION**

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### **Background**

*Antrodia cinnamomea* grows only on *Cinnamomun kanehirai* Hay (host specificity) and it is a unique fungus in Taiwan. In recent studies, the major components of *Antrodia cinnamomea* are triterpenoids whose functions were anti-inflammation and anti-cancer. So it is a valuable fungus in pharmaceutical potential. However, *Antrodia cinnamomea* grows slowly and does not obtain the fruiting body form easily under artificial cultivation.

### **Objectives**

Based on this consideration, this study modified the plant factory as the new fungus fermenter to increase the triterpenoids products.

### **Methods**

We isolated the new *Antrodia cinnamomea* strain (CUST-AC901) from the trees in the mountainous areas of *Hualien* in Taiwan. It was fermented on this new established fermenter without light, which is one type of closed plant production systems (CPPS) consisting of a thermally insulated, air conditioners, a CO<sub>2</sub> and nutrient solution supply unit. The triterpenoids were analyzed by HPLC.

### **Conclusions**

We established a new high-efficient solid-state cultivation with CUST-AC901 strain and obtained the optimal conditions (temperature at 20°C, CO<sub>2</sub> at 10%, humidity at 85%, and with 1% peptone in whole grains medium). We also obtained compositions on fruiting bodies similar to the wild ones. It only took two months and reduced the producing cost. With HPLC analysis, we confirmed that total triterpenoids products were increased by four-folds than they were under traditional culture method. To our knowledge, this is the first report to omni-functional solid-state fermenter with high triterpenoids product efficiency and low cost as well as reaching to automatic mass production without expensive automatic production equipment.



**FEMS-1318**

**Biotechnology and industrial microbiology**

**THE IMPACT OF CYTOSOLIC AND PEROXISOME LOCALIZED  
TRANSALDOLASE AND TRANSKETOLASE ON XYLOSE METABOLISM AND  
ALCOHOLIC FERMENTATION IN METHYLOTROPHIC YEAST HANSENULA  
POLYMORPHA**

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**Background**

Lignocellulose is currently considered as the most promising renewable feedstock for production of liquid biofuels. Xylose is the second most abundant sugar in nature, being a component of hemicellulose, which is in turn a part of lignocellulose. Yeasts and most of mycelial fungi can metabolize xylose by the so called xylose reductase – xylitol dehydrogenase pathway including xylose reduction to xylitol and subsequent xylitol oxidation to xylulose which is then phosphorylated by a specific kinase and enters pentose phosphate pathway (PPP). A simultaneous improvement of xylose conversion to xylulose and in the activities of PPP enzymes is required for efficient xylose utilization in different microorganisms. Xylose fermenting yeast *Hansenula polymorpha* contains in addition to cytosolic transaldolase (gene *TAL1*) and transketolase (gene *TKL1*) also peroxisomal transketolase (also known as dihydroxyacetone synthase, gene *DAS1*) and putative peroxisomal transaldolase (gene designated by us as *TAL2*).

**Objectives**

To investigate the role of cytoplasmic and peroxisomal transaldolase and transketolase in xylose metabolism and alcoholic fermentation in *H. polymorpha*.

**Methods**

Molecular-biology techniques

**Conclusions**

In the wild type strain of *H. polymorpha* overexpression of *DAS1* and *TAL2* turned out to be beneficial for xylose alcoholic fermentation. Moreover, mutants with knock out of at least one of these genes were impaired in xylose fermentation as compared to the

wild-type strain. To investigate the role of cytosolic transaldolase and transketolase in xylose metabolism of *H. polymorpha* recombinant strains overexpressing *TAL1* and *TKL1* genes were constructed. However, such strains revealed no substantial difference in ethanol production as compared to the wild-type strain.

**FEMS-1361**

**Biotechnology and industrial microbiology**

## **WOOD FOR FOOD: AVAILABLE METHODS FOR MICROBIAL HAZARD ANALYSIS**

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### **Background**

Wood is a direct food contact material used in the food industry since ancestral times. In Europe, wooden food contact surfaces are subject to the European regulation n°1935/2004, which specifies that food contact material must not interfere with foodstuff characteristics (Anonymous, 2004).

Today, no standard recovery method was defined for wooden food contact surface. Therefore, it might be important to provide an efficient method to quantify the microbial load on the wooden surfaces in direct contact with food.

### **Objectives**

The aim of this study was to compare three methods of recovering microorganisms from wooden surfaces: grinding, brushing and planing (Ismail et al., 2014).

### **Methods**

Three microorganisms, well-known as risk along the food chain, were tested: *Listeria monocytogenes*, *Escherichia coli* and *Penicillium expansum*. We chose three wooden species - pine, poplar and spruce - which are mainly used to manufacture wooden packaging. We analyzed the influence of wooden moisture content, contact time and wood timbers on microbial recovery rates.

### **Conclusions**

We identified that factors cited above influenced the microbial recovery rates from wooden surfaces. Grinding was the most reliable method with the best recovery yield: 30.1 % for *Listeria monocytogenes* on spruce and *Escherichia coli* on poplar, and 30.4% for *Penicillium expansum* on poplar. Planing was chosen to be applied to thicker wooden as cheese ripening shelves and tested, then, for analyzing the microbial load of 54 various wooden ripening shelves. We did not found common

pathogens known as risks for dairy products. Our results suggest that wood is suitable for food contact.

**FEMS-1406**

**Biotechnology and industrial microbiology**

**PROMOTER STRENGTH AND PLASMID LOSS IN SACCHAROMYCES CEREVISIAE STRAINS EXPRESSING LIPASE A FROM BACILLUS SUBTILIS**

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**Background**

GRAS status, the availability of different types of mutants and the ability to perform eukaryotic post-translational modifications, including complex glycosylation and protease processing, make *Saccharomyces cerevisiae* an ideal host for the expression of recombinant proteins. Among recombinant enzymes, lipases are used in many different industrial applications including pharmaceutical synthesis, biodiesel production, detergent formulation and food industry.

**Objectives**

In the present work, lipase A from *Bacillus subtilis* was expressed in the *S. cerevisiae* CEN.PK113-5D strain as a fusion protein with the yeast cell-wall mannoprotein Pir4. In order to compare the effect of different promoters on the levels of lipase activity secreted into the growth medium, three different YEplac195 based constructions were created, differing only in the promoter controlling the expression of the gene fusion; the original weak and constitutive PIR4 promoter, the TEF1 promoter, strong and constitutive, and the inducible GAL1 promoter.

**Methods**

The resulting three strains were grown in rich non-selective medium and the levels of secreted lipase activity and plasmid loss were determined after 48 hours of growth.

**Conclusions**

Our results show that the use of a very strong constitutive promoter is a poor strategy, since plasmid loss occurs in over 80% of cells and consequently total activity levels remain low in comparison with the strain transformed with the construction including a weaker promoter, where plasmid loss occurs in around 20% of cells. Finally, maximum levels of activity were achieved using the GAL1 promoter in conditions of induction for 24 hours after 24 hours growth in non-inducing conditions.

**FEMS-1449**

**Biotechnology and industrial microbiology**

**ENGINEERED SALMONELLA ENTERICA TYPHIMURIUM CONTROLS TUMOR GROWTH IN MURINE MELANOMA MODEL**

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**Background**

The cancer is the second most frequent cause of death. Melanoma, a malignancy that arises from melanocytes, accounts for approximately 10% of skin tumors and in recent years its incidence has increased. The mortality rate currently stands at 80%, while 5-year survival rate is less than 5%. The factors that affect cancer outcomes, such as disease recurrence, risk of second malignant neoplasms, and the late effects of cancer treatments, becomes more important. For almost 200 years has been known that bacteria have the ability to colonize solid tumors and induce tumor shrinkage. The bacteria *Salmonella enterica* Typhimurium are facultative anaerobes and has a particular promise as a cancer therapeutic because it can be manipulated and has been shown to preferentially accumulate in tumors compared with other organs after systemic injection. *S. enterica* strains can have the anti-tumor activity enhanced by genetic manipulation

**Objectives**

This study aims to evaluate the anti-tumor potential of attenuated new mutants of *S. Typhimurium* in murine models.

**Methods**

Six –week-old female C57Bl/6 mice were subcutaneously injected with  $5 \times 10^5$  B16F10 (murine melanoma). After 12 days mice were injected intratumoral with  $10^7$  CFU/mL of *S. enterica* LGBM127.

**Conclusions**

Our results showed a reduction in tumor growth and an increased survival rate in mice treated with *S. enterica* when compared to the control group (PBS). There were no signs of prostration after LGBM127 inoculation. More tests are needed, but our preliminary data demonstrate a high potential for the use of the attenuated strain of *S. Typhimurium* as antitumor agents.

**FEMS-1490**

**Biotechnology and industrial microbiology**

**PROTEIN ENGINEERING TO INCREASE THE THERMOSTABILITY AND ACTIVITY OF THE GH-10 XYLANASE FROM THERMOASCUS AURANTIACUS, BY SITE-DIRECTED MUTAGENESIS**

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**Background**

The enzymatic saccharification of lignocellulosic biomass and biobleaching paper pulp are attractive strategies for the bioprocess industry where xylanases have been heavily applied. These processes require enzymes that tolerate high stress environmental conditions such as extreme temperatures. The wild xylanase from *T. aurantiacus* (xynA) has been described as a potential enzyme for rational protein engineering studies. In the first step of this study, two no silent mutations were detected in the xynA sequence, cloned in *S. cerevisiae* strain, and it may explain the reduced expression and activity.

**Objectives**

New strategy was proposed to overcome the expression issues and enzyme improvement. First, xynA gene was rebuilt and it was used as template for constructions of mutants by site-directed mutagenesis (SDM) to increase thermal tolerance and activity.

**Methods**

For this, the original gene was amplified and cloning into pET28a. For the SDM reactions were used the Q5® SDM Kit. Primers were constructed according to the desired mutations and each one used for its changing. In the total, 13 reactions were prepared, in wich only the first two were done to the reconstruction of xynA. All the mutagenic xylanases were characterized in pH (3.5-10) and Temperature (40-80°C) by enzymatic assay.

## **Conclusions**

Regarding the optimum temperature, the enzymes are active at 65°C, and pH 5. About the stability temperature, the most of them are stable from 40°C to 65°C, and four mutants until 75°C, both in wide range of pH. These results helped the selection of the best mutagenic xylanases for future assays.



**FEMS-1495**

**Biotechnology and industrial microbiology**

## **NOVEL BIOINSECTICIDES BASED IN BACILLUS THURINGIENSIS SPORES TO CONTROL PLAGUE DISEASES IN PLANTS**

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### **Background**

The massive use of synthetic chemical agents of biocontrol (i.e. insecticides) with potential toxicity to the users and the environment pushes for the development of alternative (ecological and natural) compounds. To this respect, the use of natural and human-friendly bacteria with insecticide (i.e. larvicide) activities (biocontrol agents) represents an interesting alternative.

### **Objectives**

*Bacillus thuringiensis*, is a ubiquitous Gram-positive, spore-forming bacterium that forms soluble Vip toxins and a parasporal crystal (Cry toxin) during its growth cycle. These *B. thuringiensis* toxins are efficient for the control of certain plant-detrimental insect species among the orders *Lepidoptera*, *Diptera*, and *Coleoptera*.

### **Methods**

One hundred nineteen *B. thuringiensis* strains isolated from different agricultural regions of Argentina were characterized for Vip and crystal protein production and evaluation of toxicity against Lepidopteran insect's larvae. Finally the genomic sequencing of the 16S ribosomal subunit genes plus the PCR identification of *cry* and *vip* subclasses of selected isolates were performed.

### **Conclusions**

Several of the novel isolates were able to simultaneously control *Spodoptera frugiperda* and *Rachiplusia n̄u* larvae under laboratory and open field conditions. Interestingly, many of the isolates harbored both insecticidal genes (*vip*, *cyt*). The role of the master transcription regulator Spo0A on insecticidal protein production is presented. The understanding of the genetic network under Spo0A command opens new avenues for the development of intelligent biocontrol agents in the scope of integrated pest management for sustainable agriculture.



**FEMS-1497**

**Biotechnology and industrial microbiology**

**NOVEL BACTERIAL INOCULANTS, BASED IN BACILLUS SPORES, TO INCREASE AND PRESERVE THE NUTRITIONAL QUALITY OF ALFALFA IN LIVESTOCK FODDER**

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**Background**

Many problems associated with haymaking would be solved by reducing hay-drying time or if hay could be stored at high moisture without fungal spoilage. Bacterial inoculants are an ecological alternative for improving hay (and silage) preservation.

**Objectives**

The aim of this study was to identify, isolate and select the best spore-forming *Bacillus* strains capable of inhibiting the growth of undesirable fungus and accelerate the dehydration of forage in order to conserve its original nutritional value.

**Methods**

Seventy *Bacillus* strains were isolated from alfalfa fields of Argentina. Small bales and open field assays were prepared and treaded with each *Bacillus* strain in absence and presence of *Fusarium verticillioides*. These bales were daily monitored throughout the trial for evidence of mold growth, dehydration of alfalfa and bale organoleptic quality. Germination rates / outgrowth and biofilm / spreading were evaluated in AFGK-buffer and LB medium, respectively. Genomic identification of selected isolates was performed by sequencing of the gene coding for the 16S ribosomal RNA. Five isolates (initially identified as *B. subtilis*, *B. pumilus* and *B. amyloliquefaciens*) showed a broad spectrum of fungal control and an accelerated dehydrating capacity of alfalfa that improved the retention of its nutritional values in the forage. Viable spores were showed to be needed for these effects and the capacity of alfalfa colonization by mechanisms of biofilm formation and spreading were identified.

**Conclusions**

The novel *Bacilli* presented in this work, and the understanding of the displayed molecular mechanisms, are suitable for the production of novel inoculants to conserve forages of high nutritional value.



**FEMS-1500**

**Biotechnology and industrial microbiology**

## **GENE DISRUPTION ANALYSIS FOR ETHANOL PRODUCTION IN GLYCEROL FERMENTATION IN GLYCEROL-ASSIMILATING KLEBSIELLA VARIICOLA AND ITS METABOLIC CHANGES**

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### **Background**

The gram-negative enteric bacterial genus *Klebsiella* can utilize glycerol as a sole carbon source and is known to produce some valuable metabolites, such as ethanol and 1,3-propanediol (1,3-PD). Previous studies, we have obtained streptomycin-resistance *K. variicola* mutant, strain TB-83D, as an ethanol producer from glycerol, and have demonstrated that it showed higher ethanol production than parent strain (Suzuki et al. Bioresour. Technol. 2015.). To further increase ethanol productivity, metabolic changes and inhibition of byproduct production by gene disruption were required.

### **Objectives**

To further improve ethanol production, the aim of this study is to increase gene induction efficiency for gene disruption and to suppress byproduct production by gene disruption.

### **Methods**

Gene disruption was performed by using modified Red recombination method. Metabolite concentrations were measured by HPLC (Waters alliance 2695) and F-kit D-/L-lactic acid (Boehringer Mannheim).

### **Conclusions**

Since strain TB-83D produced mainly D-lactate under optimum conditions, we considered that suppression of D-lactate production is effective in improving ethanol production. To suppress D-lactate production, we disrupted lactate dehydrogenase gene (*ldhA*) by Red recombination. Gene disruption efficiency of *Klebsiella* species is low than that of other *Enterobacteriaceae* family. Therefore, we modified the conventional Red recombination, and succeeded in improvement of gene disruption efficiency. Although *ldhA* disruptant did not produce lactate in glycerol fermentation, ethanol production increased than TB-83D. Metabolic flux analysis and metabolic changes are in progress to optimize the ethanol production process, the results of which will be described in detail in due course.



**FEMS-1502**

**Biotechnology and industrial microbiology**

**DESIGN OF SACCHAROMYCES CEREVISIAE PRODUCING CELLULOSOME-BASED CELLULOLYTIC ENZYME COMPLEXES FOR LIGNOCELLULOSIC BIOMASS UTILIZATION**

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**Background**

Heterogeneous structure of lignin imparts plants with structural rigidity and also serves to protect cellulose and hemicellulose from degradation. Thus, prior to fermentative production of ethanol from the cellulose by yeast strains, the materials are degraded and hydrolyzed to release monomeric sugars.

**Objectives**

In this study, designer cellulosome was assembled in yeast *Saccharomyces cerevisiae* for utilizing of cellulose as the substrate. For utilizing of cellulose part in lignocellulosic biomass by simultaneous saccharification and fermentation, a recombinant scaffolding protein from *Clostridium cellulovorans* and a chimeric endoglucanase E from *Clostridium thermocellum* were assembled as complex system. Compared to the results for single subunit, assembly of cellulosome-based enzyme complexes caused a noticeable increase such as 2.1-fold-higher in the level of enzyme activity. The resulting strain was able to ferment cellulose part in pretreated barley straw into ethanol with the aid of beta-glucosidase A from *C. thermocellum*. In the fermentation assay at 10 g/L initial substrate, approximately 2.1-folds higher ethanol than that of wild type was produced.

**Methods**

The use of complexed enzyme systems is one of the strategies for effective lignocellulosic biomass hydrolysis. Enzyme complexes were formed via the interaction of a dockerin domain with cohesin modules in the scaffolding protein.

**Conclusions**

Accelerating the biological degradation of lignocellulosic materials will benefit from the development of useful recombinant enzymes with hydrolysis ability. In future research, construction of designer enzyme complexes containing other lignin degrading enzymes could be used to develop biocatalysts that can completely degrade lignocellulose into single sugars.

**FEMS-1521**

**Biotechnology and industrial microbiology**

**CHARACTERIZATION OF PHYSIOLOGICALLY ACTIVE COMPOUNDS AND CO<sub>2</sub> REDUCTION IN RHODOBACTER SPHAEROIDES MUTANT STRAINS**

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**Background:**

The purple nonsulfur photosynthetic bacteria, *Rhodobacter sphaeroides*, are capable of growing under a variety of environmental conditions. Particularly this microorganism can use carbon dioxide as a carbon source to grow. In addition, this bacterium also has the capability to produce all kinds of the polyphenol, nucleic acid material, vitamin, bio-active substances and polyhydroxybutyrate (PHB). Therefore, this study focused on analyzing the properties of organic compounds and reduction of CO<sub>2</sub> in *R. sphaeroides* mutant strains.

**Objectives:**

In this study, relying on the ability to produce physiologically active compounds (PACs) and reduce CO<sub>2</sub>, four different chemically mutated strains: *R. sphaeroides* KCTC 1434, MBTLJ-8, MBTLJ-13 and MBTLJ-20 were screened in order to select the most optimal strain.

**Methods:**

*R. sphaeroides* KCTC 1434, MBTLJ-8, MBTLJ-13 and MBTLJ-20 that are all chemically mutated strains, were used in this study. To be more precise, the comparison among these strains in terms of their ability to produce physiologically active compounds (PACs) and reduce CO<sub>2</sub> was carried out.

**Conclusions:**

The result showed that the mutant strain MBTLJ-8 performed the best ability of CO<sub>2</sub> fixation. In addition, it also showed that the CO<sub>2</sub> reduction and cell growth of mutant strains enhanced under the blue colored light emitting diode (LED) conditions. Therefore, our data indicated that the wave length of special light probably affected the cell growth, CO<sub>2</sub> reduction and organic compounds production of mutant strains.



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**FEMS-1525**

**Biotechnology and industrial microbiology**

## **EFFECT AND ANALYSIS OF RHODOBACTER SPHAEROIDES EXTRACT UNDER VARIOUS CULTURE CONDITIONS**

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### **Background:**

*Rhodobacter sphaeroides* is a gram negative purple nonsulfur photosynthetic bacterium, facultative photoheterotrophic bacterium capable of growing phototrophically or chemotrophically as either a heterotroph or lithotroph in the presence or absence of O<sub>2</sub>, depending on the energy source. In addition, it owns capability to produce all kinds of amino acids, nucleic acid materials, vitamins, bio-active substances, polyphenol and polyhydroxybutyrate (PHB).

### **Objectives:**

In this study we focus on analyzing the properties of organic compounds and reduction of CO<sub>2</sub> in *R. sphaeroides* under various culturing conditions. Moreover, it is another goal of this study to confirm the effect on promoting growth of other microorganisms caused by the complement of *R. sphaeroides* extract in various culture conditions.

### **Methods:**

*R. sphaeroides* KCTC1434 were used in this study. More precisely, the analysis of growth promoting effect of the extract of *R. sphaeroides* was conducted under various culture conditions.

### **Conclusions:**

Our data indicated that addition of the *R. sphaerodes* extract into culture media accelerated the growth of microorganisms. Therefore, there was a strong evidence showing that the *R. sphaerodes* extract has multifunctional potential to increase the physiological activity of other organisms. It is, thus, determined as an enhanced material which is able to force to activate the growth promotion of other organisms.

**Acknowledgments:** This work was carried out with the support of “Cooperative

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**FEMS-1551**

**Biotechnology and industrial microbiology**

**CLONING AND HETEROLOGOUS EXPRESSION OF ACID AND THERMOSTABLE B-GLUCOSIDASE IN PICHIA PASTORIS X-33 FROM THERMOASCUS AURANTIACUS RCKK**

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**Background**

Thermostable cellulases offer several advantages like higher rates of substrate hydrolysis, lowered risk of contamination and increased flexibility with respect to process design. The production of cellulases from a thermophilic fungus identified as *Thermoascus aurantiacus* RCKK (Acc. No. JN676149) has been attempted.

**Objectives**

In order to increase production level of cellulases and eventually economize the process, heterologous expression of these genes in *Pichia pastoris* was carried out.

**Methods**

$\beta$ -glucosidase gene from *T. aurantiacus* RCKK was cloned and overexpressed in *P. pastoris* X-33.

**Conclusions**

A 2.5 fold higher production of recombinant  $\beta$ -glucosidase was achieved at shake flask level in comparison to natively expressed enzyme. Expression of  $\beta$ -glucosidase of about 96kDa was confirmed by SDS PAGE, western blot and zymogram analysis. The purified recombinant  $\beta$ -glucosidase was found to be stable in wide range of pH (3.0-8.0), temperature (up to 50% activity after 3 h at 80°C) and tolerant to the presence of ionic liquid (1-ethyl-3-methylimidazolium acetate [C2mim][OAc]. These properties make the recombinant enzyme an important catalyst for carrying out an efficient hydrolysis of cellulose to sugars and enzymatic deinking of recycled paper pulp. This will eventually open up potential of this enzyme.

**FEMS-1567**

**Biotechnology and industrial microbiology**

## **RAPID DEGRADATION OF LIGNOCELLULOSIC BIOMASS**

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### **Background**

The main obstacle to lignocellulose bioconversion is the presence of lignin and crystalline cellulose in plant cell wall. However, major factors have not been definitely determined between lignin and crystalline cellulose in enzymatic lignocellulose recalcitrance. The correlative information promotes a deeper understanding of lignocellulose bioconversion characteristics.

### **Objectives**

Our aim is to determine the major rate-limiting factor in the hydrolysis of popping pre-treated rice straw (PPRS) by examining cellulase adsorption to lignin and cellulose, re-hydrolysis, and amorphogenesis.

### **Methods**

Lignin was prepared from PPRS by enzyme treatment until complete hydrolysis of carbohydrates and used to measure enzyme adsorption. Re-hydrolysis experiments were repeatedly performed with 15.75 or 31.50 FPU g<sup>-1</sup>-biomass cellulase for 1 or 3 hour until complete hydrolysis of 1 or 2% PPRS. Amorphogenesis of PPRS was carried out with 80% phosphoric acid on ice for 1 hour.

### **Conclusions**

Lignin isolated from PPRS adsorbed 20% of enzyme loading from 3.15-31.50 FPU g<sup>-1</sup>-biomass, adsorbing low levels of exoglucanases, endoglucanase and xylanase. Marked inhibition by cellulose structural effect was occurred during re-hydrolysis steps. Amorphogenesis of PPRS resulted in twofold higher cellulase adsorption and increased the yield of the first re-hydrolysis step from 13% to 46%. The total yield was increased to 84% in 3 h. These results provide strong evidence that cellulose structure, not lignin, has a major effect on the enzymatic hydrolysis of lignocellulose.

**FEMS-1570**

**Biotechnology and industrial microbiology**

## **BIOLOGICAL RECYCLING OF POLY(L-LACTIDE) (PLA) BY MICROBIAL ENZYME**

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### **Background**

Poly(L-lactide) (PLA) is a biodegradable polymer. It is synthesized from lactic acid that is produced by microbes from agricultural products. It has much attention since it plays an important role to resolve the global warming problem. In general, PLA is polymerized commercially by chemical processes. However, these processes need severe conditions, i.e. high temperature, high solvent and catalyst loading. Recently, biological polymerization methods have been presented using lipases for the bio-catalytic reaction.

### **Objectives**

This work we aimed to apply the biological process for recycle of PLA.

### **Methods**

Using a protease produced by *Actinomadura keratinolytica* strain T16-1. 1 g/L PLA powder was incubated at 77 °C for 24 h using an enzyme concentration of 100 mg/L. As a result, 750 mg/L lactic acid was obtained and used as substrate for re-polymerization of PLA. This was done by using commercial lipase under 0.1 vvm nitrogen atmosphere at 60 °C for 6 h.

### **Conclusions**

**Oligomers with molecular weight of 450 (n=4) were obtained. This is the first report to demonstrate the recycling of PLA wastes biologically.**

**FEMS-1584**

**Biotechnology and industrial microbiology**

**OPTIMIZATION OF EXTRACELLULAR LIPASES PRODUCED BY NEWLY ISOLATED BACILLUS SAFENSIS STRAIN PSR**

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**Background**

Lipases are an important enzyme for many biotechnological applications due to they are biocatalysts which have an activity at mild condition. Recently, bacterial lipase was very interesting because it can be used for re-polymerization of biodegradable plastics such as poly(L-lactide).

**Objectives**

The present work was aimed to screen lipase-producing bacteria from oil-contaminated soil in South of Thailand and study the optimization of medium composition for lipase-production.

**Methods**

Lipase-producing bacteria with 662 strains were isolated from 100 soil samples according to opaque zone formation on Tween 80 agar. Strain PSR exhibited the highest lipase activities in both agar plate and production medium. It was identified as *Bacillus safensis* strain PSR based on 16S rRNA gene sequencing. Moreover, the factors influencing lipase production were investigated in shake flask such as carbon and nitrogen sources, pH, temperature and incubation time. The maximum enzyme activity 63.86 U/ml was obtained by using 0.5% (w/v) rice bran oil and 0.12 % (w/v) yeast extract as carbon and nitrogen sources, respectively, at pH 6.85, 37°C and 60 hours cultivation.

**Conclusions**

The study provides a high bacterial lipase production which is a good candidate to apply for recycle of biodegradable plastic waste in the near future.

**FEMS-1590**

**Biotechnology and industrial microbiology**

**RECONSTRUCTION OF A CORE METABOLIC NETWORK RELATED TO MEDIUM-CHAIN-LENGTH POLYHYDROXYALKANOATE PRODUCTION BY PSEUDOMONAS SP. LFM046 FROM CARBOHYDRATES.**

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**Background**

Medium-chain-length polyhydroxyalkanoate (PHA<sub>MCL</sub>) are biodegradable elastomeric polymers produced from renewable resources. *Pseudomonas* sp. LFM046 is a PHA<sub>MCL</sub> producer from carbohydrates sources, presenting a better performance than the most studied *Pseudomonas putida* KT2440.

**Objectives**

In this work, based on 16S rDNA sequences, *Pseudomonas* species phylogenetically close to *Pseudomonas* sp. LFM046 were identified and their sequenced genomes were searched for genes involved in carbohydrates catabolism and PHA biosynthesis, allowing reconstructed a core metabolic network. To confirm this network, the whole genomic DNA of *Pseudomonas* sp. LFM046 was sequenced.

**Methods**

For this, genomic DNA was extracted and fragmented using Covaris. Paired-end sequencing library was constructed using TruSeq DNA PCR-Free LT Sample Preparation Kit. The library quantification was performed using QUBIT, **Agilent Bioanalyzer** and real time PCR. The library was sequenced on an Illumina MiSeq sequencer.

**Conclusions**

*De novo* assembly generated 34 contigs using 3,032,982 reads, and the average size of 50% of the contigs (N50) was 640,128 bp, with the maximum size of 1,038,386 bp. The genome size was estimated at 5,970,318 bp. All genes involved in carbohydrate metabolism and PHA biosynthesis previously detected in other *Pseudomonas* species were also identified and annotated in the genome of *Pseudomonas* sp. LFM046 using RAST software. It includes genes from Entner-Doudoroff (ED) and pentose phosphate (PPP) pathways, tricarboxylic acid (TCA) and glyoxylic acid cycles (GAC), *de novo* fatty acids (FAB) and PHA biosynthesis. Thus, a core metabolic network of *Pseudomonas* sp. LFM046 was reconstructed and is going to be used to determine flux distribution based on exchange flux measurements.

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**FEMS-1616**

**Biotechnology and industrial microbiology**

## **GEN SPECIFIC QUANTIFICATION OF BACTERIAL CELLS IN ACTIVATED SLUDGE SYSTEMS**

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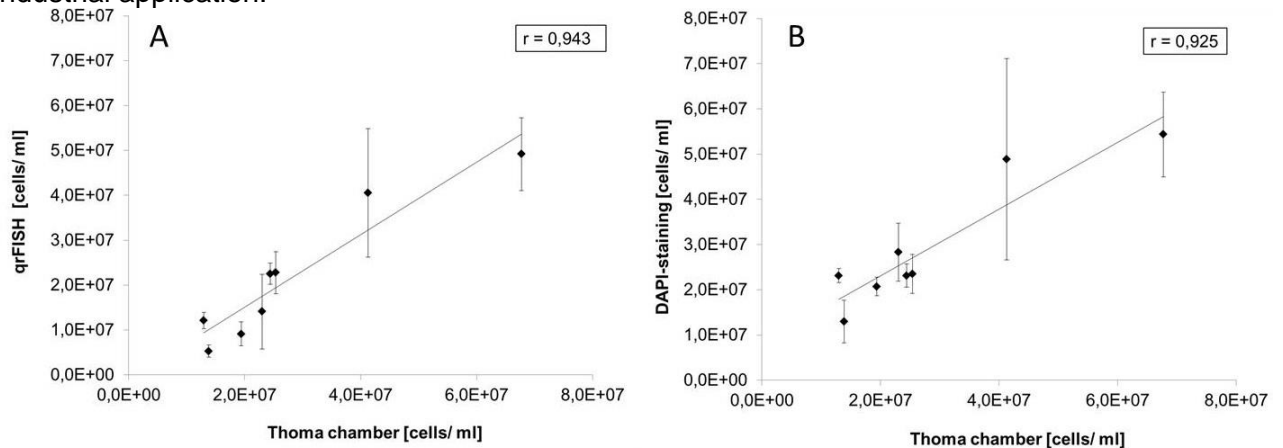
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### **Background**

Modern waste water treatment plants utilize activated sludge systems to reduce soluble compounds in waste water. The diversity of the biocenosis is primarily affected by the composition of the treated waste water. Especially the distribution of carbon and nitrogen leads to a characteristic diversity. Information about the specific abundance of essential microorganisms can be used to improve the plant operation and stability of the biological degradation.

### **Objectives**

While available quantitative methods like MPN (most probable number) and qPCR (quantitative polymerase chain reaction) are time consuming, this work led to the development of a rapid and quantitative variation of the fluorescence in situ hybridisation (qrFISH). The main objective was to design a method for a daily industrial application.



### **Methods**

Fluorescence in situ hybridisation was carried out according to Nielsen (2009). Fluorescence particles were added to the sludge samples and used as a reference for quantification. Thereby the unknown loss of biomass throughout the FISH process could be compensated.

## **Conclusions**

The current results showed a strong correlation between cell counts before and after the hybridisation process. Figures 1 shows the comparison of quantification results by fluorescence microscopy and conventional counting in a Thoma chamber. The experiments were conducted with enriched cultures of bacterial cells. The primary limitation of quantitative FISH was overcome, and the results were achieved in the targeted time frame of 8 hours.

**FEMS-1617**

**Biotechnology and industrial microbiology**

**CHARACTERIZATION OF RECOMBINANT LACCASE (BPCOTA) FROM  
BACILLUS PUMILUS MK001 AND ITS POTENTIAL FOR PHENOLICS  
DEGRADATION**

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**Background**

Bacterial laccases are very less explored despite their superior properties such as high thermostability and wide pH stability. In the present study, Putative laccase (CotA) from *Bacillus pumilus* MK001 has been cloned and expressed in *E. coli* BL21(DE3).

**Objectives**

Hyper-production of recombinant bacterial laccase was carried out to utilize its potential in bioconversion of phenolics.

**Methods**

BPCotA was expressed in *E. coli* BL21(DE3) and purified to homogeneity. Purified enzyme was characterized for its thermal and pH stability. The 3D model of BPCotA was constructed based on the crystal structure of *B. subtilis* CotA (PDB Id: 1GSK) by homology modelling (Modeller 9.11). Modeled structure was refined and validated for stereochemical quality using PROCHECK, ERRAT, Verify 3D and PROSA servers. *In silico* interaction between enzyme and phenolics was studied out to predict *in vitro* phenolics degradation ability of the recombinant enzyme.

**Conclusions**

The recombinant BPCotA was purified with ~82% recovery exhibiting ~230 IU/mg specific activity. The recombinant BPCotA retained more than 50 % of the original activity after incubating at 80°C and 90°C for 90 min and 30 min, respectively, and more than 80% of its activity in the wide pH range 5.0-9.0 for 240 h. Increase in temperature from 30-90°C resulted in significant changes in secondary structure as predicted by far UV thermal CD spectra. Docking analysis showed that both ferulic acid and vanillin bind in the vicinity of predictive active site of recombinant protein. Further, *in vitro* action of the enzyme on phenolics resulted in bioconversion of both ferulic acid and vanillin.



**FEMS-1680**

**Biotechnology and industrial microbiology**

**THE EFFECT OF METHANE DIGESTED SLURRY FOR ETHANOL PRODUCTION FROM GLYCEROL BY KLEBSIELLA VARIICOLA STRAIN TB-83D.**

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**Background**

Biodiesel fuel (BDF) waste contains large amounts of crude glycerol as a by-product, and its treatment is an environmental problem. In this study, it was attempted to produce bio-ethanol from crude glycerol. In previous study, we isolated *Klebsiella variicola* strain TB-83D that could produce ethanol from glycerol. This strain has high potential of ethanol production, and its production was 32 g/L by using yeast extract (YE) as a nutrient. However, YE is very expensive, so it is not suitable for an industrial use.

**Objectives**

The objective of this study is to decrease its culture cost. And we attempted to use methane fermentation digested slurry (MFDS). MFDS is residue of methane fermentation and its cost is very low.

**Methods**

The experiment was conducted by 1 L fermenter at 25°C and the culture pH was maintained at around 8.0 with 6 N NaOH. Basal medium consist of 50 g/L of glycerol and 600 g/L of MFDS is used for ethanol production by strain TB-83D.

**Conclusions**

The ethanol production was decreased to 17 g/L by replacing YE with sterilized MFDS. However, we think that this result seems to be a promising start to establish a low cost ethanol production from BDF waste. Next, we used non-sterilized MFDS to reduce sterilizing cost. Unfortunately, ethanol production was decreased but 1,3-propanediol and acetate production were observed. It may be caused by other bacteria survived in the MFDS. Now we are trying to optimize the culture condition that strain TB-83D is dominant when using non-sterilized MFDS.

**FEMS-1684**

**Biotechnology and industrial microbiology**

**CAT8 GENE IS INVOLVED IN REGULATION OF XYLOSE ALCOHOLIC  
FERMENTATION IN THE THERMOTOLERANT YEAST HANSENULA  
POLYMORPHA**

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**Background**

Efficient alcoholic fermentation of hexose and pentose sugars by yeast is essential to achieve maximal ethanol yield from lignocellulosic hydrolyzates. It is known that *CAT8* encodes carbon source-responsive transcriptional activator that controls expression of genes involved in gluconeogenesis. Deletion of *CAT8* in the non-conventional yeast *Pichia guilliermondii* activated alcoholic fermentation.

**Objectives**

The aim of this work was to isolate mutants of *H. polymorpha* with knock out of the gene *CAT8* and study their alcoholic fermentation. The strains were planned to be constructed in the backgrounds of the wild-type strain and of the mutant with elevated ethanol production from xylose due to overexpression of the genes encoded the key enzymes involved in xylose metabolism (xylose reductase, xylitol dehydrogenase, xylulokinase) and resistance to 3-bromopyruvic acid (glycolysis inhibitor).

**Methods**

The cassettes for *CAT8* gene deletion was constructed by used of homologous recombination method with gene *HphNT1* conferring resistance to hygromycin B as the selective marker. Fermentation was carried out at a temperature of 45°C with limited aeration. Concentrations of ethanol in the medium were determined using alcohol oxidase/peroxidase-based enzymatic kit 'Alcotest'.

**Conclusions**

Deletion of *CAT8* gene did not have effect on glucose alcoholic fermentation. At the same time, constructed knock out strains showed an improved xylose alcoholic fermentation relative to the parental strains. The  $\Delta cat8$  strains isolated from the best available ethanol producer accumulated up to 12.5 g of ethanol/L at 45°C after 3 day fermentation without correction for evaporation. This is the 30 fold increase as compared to the wild-type strain



**FEMS-1703**

**Biotechnology and industrial microbiology**

**CHIMERIC ROTAVIRUS AND HEPATITIS A VIRUS-LIKE PARTICLES INDUCE VIRUS-SPECIFIC HUMORAL AND CELLULAR IMMUNE RESPONSES IN MICE**

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**Background**

Rotavirus and hepatitis A virus (HAV) are transmitted by the fecal-oral route and continues to be endemic throughout the world. Virus-like particles (VLPs) is safe and represents an economical approach to combined multivalent vaccine development.

**Objectives**

D2/VP7 VLPs demonstrated excellent combined vaccine candidate and may lead to the development of a commercialized vaccine worldwide for preventing both rotavirus and HAV.

**Methods**

In the current study, we evaluated the humoral and cellular immune responses in mice vaccinated with hepatitis A virus/rotavirus VLPs vaccine manufactured in methylotrophic yeast *Pichia pastoris* strain GS115. The VLPs were comprised of the hepatitis A virus D2 epitope and rotavirus  $\Delta$ VP7 proteins. BALB/c mice vaccinated with chimeric VLPs.

**Conclusions**

VLPs produced high levels of neutralizing antibodies in sera against rotavirus and HAV. Induction of the secretion cytokines were detected from anti sera. In addition, splenic CD4+ and CD8+ T cell accessory molecules increased and produced cytokines after vaccination.



**FEMS-1745**

**Biotechnology and industrial microbiology**

**EXOPOLYSACCHARIDES PRODUCED BY MEMBERS OF ACIDOBACTERIA  
PHYLUM AND ITS PROPERTIES**

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**Background**

Biopolymers are becoming more and more attractive for the industry due to their biological/natural origin, sustainability, biodegradability and low toxicity. This results in an increased demand for novel biopolymers, including exopolysaccharides (EPS) for different biotechnological purposes. It is worth to mention a broad spectrum for applications ranging from human health, over food and fodder production to chemical industry and environmental applications.

The ability to synthesize EPS is quite common among bacteria. Thus, as described exopolysaccharides differ in their physical and chemical properties, it may be surprising that only few of those products were successfully commercialized.

**Objectives**

In our studies we focused on *Acidobacteria*, highly divers and environmentally widespread but not well studied bacteria phylum, as a source of valuable compounds. The ability to synthesize EPS has been previously reported for some members of this phylum. However, its chemical composition and properties were not investigated up to date.

Taking into consideration a total lack of information on acidobacterial EPS, the aim of the present work was first of all to gain insight into the nature of those polymers and second to explore some of the characteristics potentially novel and superior for industrial applications.

**Methods**

We thus, focused on isolation, purification and physico-chemical characterization of EPS. Additionally, the stability of emulsions formed with selected oils and hydrocarbons.

**Conclusions**

The study proved different composition of EPS produced by closely related bacteria translating into differences in its properties but also a high stability of emulsions suggesting potential of EPS as stabilizing agents and/or bio-emulsifiers.

**FEMS-1766**

**Biotechnology and industrial microbiology**

**DETERMINATION AND CHARACTERIZATION OF PHYTASE FROM A NEWLY ISOLATED BACILLUS SP.**

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**Background**

Phytate is the primary storage of phosphate in plants, and abundant in legumes, cereals and nuts. However, phytate make a polyanionic chelating agent which reacts with proteins, amino acids and divalent cations in humans and animals. Resulting in malnutrition and several health problems. Phytase is used as additive for degradation of antinutritional phytate and the enzyme is desired to be highly thermostable for it to withstand feed formulation conditions.

**Objectives**

The production of phytase is aimed from a new microbial source that was isolated from natural sources.

**Methods**

12 different soil samples taken from barn and coop surroundings were used for isolation of microorganism. To identify microorganism, we used 16srRNA analysis. For phytase production, strains were inoculated to Phytase-Screening Medium for 3d at 30°C. After phytase activity detection, enzyme was purified by ammonium-sulphate precipitation and DEAE-Sepharose. Samples were analyzed by SDS-PAGE.

**Conclusions**

Isolate (K12) was found *Bacillus subtilis* according to 16S rRNA sequencing. Several production parameters were optimized in order to ensure high enzyme production. In production medium, glucose was founded as the most suitable C-source and ammonium nitrate (NH<sub>4</sub>NO<sub>3</sub>) was found to be most suitable N-source. In the presence of glucose, NH<sub>4</sub>NO<sub>3</sub>, sodium phytate and stirred speed (150 rpm), 72 hours incubation at 30°C were the most suitable conditions for phytase production. Phytase obtained from optimized production conditions, at pH 6.5 at 60°C and enzyme substrate ratio of 1:9 has the highest activity. MgCl<sub>2</sub>, CuCl<sub>2</sub>, CoCl<sub>2</sub>, ZnCl<sub>2</sub> solutions and EDTA causing a loss of phytase activity whereas CaCl<sub>2</sub> solution has been found to be effective as an activity enhancer.

**FEMS-1767**

**Biotechnology and industrial microbiology**

**APPLICATION OF TYROSINASE PPO2 AS A BIOMARKER FOR THE FITNESS EVALUATION OF GENOME-REDUCED *SACCHAROMYCES CEREVISIAE* CELLS**

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**Background**

In recent years, the availability of explosive whole-genome information has thoroughly reshaped the strain reconstruction studies for potential industrial applications. Consequently, the concept of minimum genome factories (MGFs) was proposed, which can be defined as recombinant strains whose metabolism have been streamlined to the optimal minimal subset applicable to specific utilities. The construction of MGF generally involves multiple large-scale genomic manipulations leading to a highly reduced genome. To this end, a wide range of genome engineering systems have been developed and applied in various microorganisms. One new issue raised thereafter is the fitness assessment of numerous resulting genome-reduced cells.

**Objectives**

Establish a simple and efficient system to evaluate the genome-reduced *Saccharomyces cerevisiae* chassis as platform for the production of value-added secondary metabolites.

**Methods**

A set of *S. cerevisiae* cells harboring large-scale deletions of different chromosomal regions was obtained using the recently developed *mazF*-based scarless genome engineering system. On the other hand, the coding sequence of tyrosinase *PPO2* from *Agaricus bisporus* was amplified and cloned into yeast expression vector pLC12. The recombinant plasmid was transformed into all deletion cells by lithium acetate method. The product of *PPO2* can convert intracellular tyrosine into colored substance melanin, which can be measured through spectral.

**Conclusions**

The tyrosine formation of yeast deletant cells can be readily deduced from the formation of melanin and this will be helpful in strain optimization for biosynthesis of valuable chemical based on tyrosine.

**FEMS-1786**

**Biotechnology and industrial microbiology**

**AMYLASES OF THE POLYEXTREMOPHILIC BLACK YEASTS AUREOBASIDIUM SPP. AND THEIR BIOTECHNOLOGICAL POTENTIAL**

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**Background**

Four varieties of *Aureobasidium pullulans* (Dothideales, Ascomycota), a cosmopolitan melanised yeast-like fungus with good stress tolerance, were recently redefined as four separate species: *A. pullulans*, *A. subglaciale*, *A. melanogenum*, and *A. namibiae*. These species inhabit various environments, including those with temperature and pH extremes, high UV radiation and high salt concentrations – and are thus considered to be polyextremotolerant. *Aureobasidium* spp. are biotechnologically important organisms, mainly due to their production of the extracellular polysaccharide pullulan, antimycotic aureobasidin A, siderophores, and various extracellular enzymes. *Aureobasidium pullulans* is also used in agriculture as a biocontrol agent while *A. melanogenum* is implicated in human disease. The recent sequencing of the genomes of the four above listed *Aureobasidium* species opened new possibilities for numerous novel applications in biotechnology, medicine, pharmacy, food industry, biofuel production, and in other fields.

**Objectives**

The aim of the study was to develop a yeast strain with good amylolytic activity under normal and stressful (industrially relevant) conditions.

**Methods**

In this study, we have cloned all alpha-amylase and glucoamylase encoding genes from the *Aureobasidium* species, used in the genome sequencing, into *S. cerevisiae*. The amylolytic performance of the transformants at different temperatures, low water activity and other relevant conditions was then evaluated. The best amylases were expressed in an industrial *S. cerevisiae* strain.

**Conclusions**

Yeast strains with good amylolytic activity under the conditions of increased salt concentration, low water activity, low temperatures and other relevant conditions were developed.

FEMS-1803

Biotechnology and industrial microbiology

## IMPROVING THE PRODUCTION OF HYDROPHOBIN HFBI FROM *TRICHODERMA REESEI* ON AN INNOVATIVE BIOFILM SOLID PACKING SYSTEM FERMENTOR

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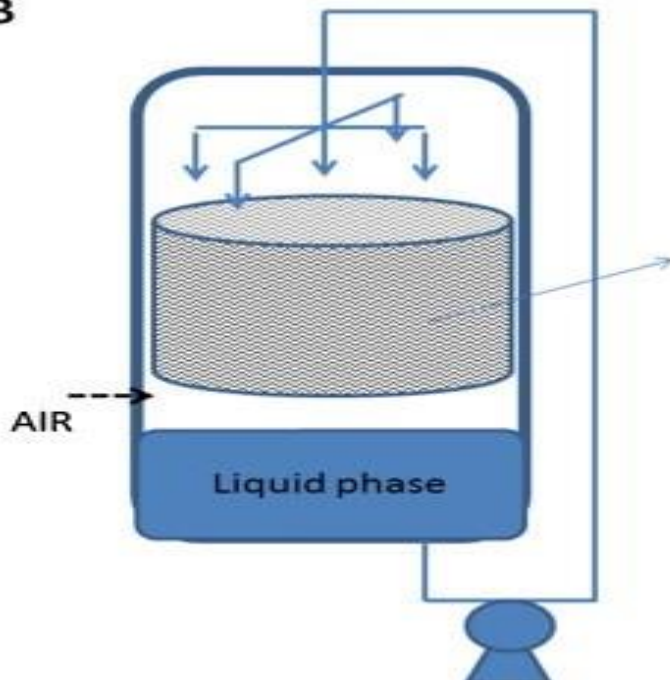
### Background

Hydrophobins are amphipatic proteins with high surface activity produced by ascomycetes and basidiomycetes. their interfacial properties make them very interesting to use them as emulsifiers and stabilizators for the food industry. However a major drawback is the low production of protein without the use of genetic techniques. Here we report the use of an innovative biofilm reactor which uses a metallic packing system as a substrate to grow on.

### Objectives

- Increase the production of hydrophobin HFBII from *Trichoderma reesei* using a biofilm reactor system

**B**



- Design of a reproducible, easy to scale up and relatively cheap method to increase the production of a fungal hydrophobin from *Trichoderma reesei*.

### Methods

- Fungal strain and culture medium: *Trichoderma reesei* MUCL 44908 were

inoculated in 20L of culture medium supplemented with glucose.

-Bioreactor set up: Along with the fermentation under biofilm system a submerged fermentation was also carried out in order to compare the increase in the production. In the case of the biofilm, the reactor was filled with a metal structured packing (Sulzer, Chemtech) where a peristaltic pump recirculated the medium evenly over the top of the packing system, this cycle was continued for the total time of the fermentation.

### **Conclusions**

- The protein production was 2.16 times higher that when submerged culture was used.
- The total weight of the fungal biomass was 33% higher that in submerged culture.
- The separation process between medium culture and fungal biomass was greatly enhanced, more than 99% of the biomass was attached to the biofilm



**FEMS-1886**

**Biotechnology and industrial microbiology**

## **MICROBES AS TOOLS FOR MYCOTOXIN DEGRADATION**

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### **Background**

Mycotoxins are secondary metabolites produced by several fungi. These toxic chemicals play a major role as feed and food contaminants. They can severely impact animal health, which is associated with high costs in livestock industry. Approximately 25% of all commodities worldwide are polluted by mycotoxins. The most prominent mycotoxins in contaminated food are Aflatoxins (hepatotoxic and cancerogenic), Deoxynivalenol (immunosuppression, protein synthesis inhibition), Zearalenone (infertility, abortion), Fumonisin (hepatotoxicity, nephrotoxicity) and Ochratoxin A (Neurotoxicity and cancerogenicity).

### **Objectives**

To counteract this problem, several strategies are applied, which involve prevention of fungal contamination during production and storage, followed by physical, chemical, and biological methods to reduce mycotoxin levels.

### **Methods**

Biological methods such as the use of microbial detoxifiers have the highest potential for feed decontamination. Bacteria capable of metabolizing mycotoxins allow decontamination and preserve nutritional value at the same time. To identify microbial detoxifiers, contaminated soil samples are cultured with a mycotoxin of interest to enrich, potential degrading strains. This eases the screening of single colonies for detoxification, which is a time consuming procedure and associated with extensive labor. Once the organism responsible for detoxification has been isolated and identified, further experiments are necessary to ensure the organism is fermentable, non-pathogenic and can be applied as direct feed additive.

### **Conclusions**

To date several detoxifying microbes have been found and characterized. An example for such a detoxifier is the strain BBSH 797 which degrades trichothecenes and is the only detoxifying feed additive on the market.



**FEMS-1895**

**Biotechnology and industrial microbiology**

**PENIOPHORA SP. CBMAI 1063 AS LACCASES PRODUCER: A MARINE ADAPTED STRAIN**

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**Background**

Laccases are oxidoreductases enzymes that use molecular oxygen to oxidize phenolic and non-phenolic compounds. Laccases' applications are related to bioremediation of hazards compounds, biobleaching and mill water treatment on pulp/paper industry, as component for phenolic detection, and for the synthesis of new "eco-friendly" polymers, antibiotics and cosmetics. Although its importance, it is still lacking ways to produce high amounts of laccases. Therefore, the search of new laccases and new producers are highly important for its commercial application. The marine-derived basidiomycete *Peniophora* sp. CBMAI 1063, isolated from the sponge *Anphimedon viridis* (Brazillian coast) is able to produce significant amounts of laccase. Previous studies showed that the laccase produced by this fungus has a good pH/thermal activity range and metallic ions resistance.

**Objectives**

To evaluate the laccase production by *Peniophora* sp. CBMAI 1063 under the presence of CuSO<sub>4</sub> and saline conditions.

**Methods**

The fungus was cultivated in 50 mL of optimized medium under different concentrations of CuSO<sub>4</sub> and artificial seawater (ASW) during 7 days at 140 rpm and 28 °C. Laccase was quantified spectrophotometrically using ABTS as substrate.

**Conclusions**

The highest laccase activity (around 2000 U L<sup>-1</sup>), was obtained in the presence of 2 mM of CuSO<sub>4</sub> and 65% (v/v) of ASW. There was no laccase activity in the absence of ASW, suggesting its importance in fungal metabolic pathway. *Peniophora* sp. CBMAI 1063 has shown a great potential to be a laccase producer under saline conditions. The produced laccase are being characterized and its genes are being studied for heterologous expression.

**FEMS-1922**

**Biotechnology and industrial microbiology**

**EFFECTS OF ENVIRONMENTAL CONDITIONS ON THE EXOPOLYSACCHARIDE PRODUCTION OF A NOVEL BACILLUS STRAIN ISOLATED FROM SOIL**

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**Background**

Microbial exopolysaccharides (EPS) have wide range of applications in food, pharmaceutical and other industries due to their stabilizing, gelling and texturizing properties. Various EPSs from different microorganisms including yeast and bacteria have been determined. They can be either homopolysaccharides (e.g. cellulose, dextran, pullulan, levan, curdlan) or heteropolysaccharides (e.g. xantan, gellan).

**Objectives**

In this study, exopolysaccharide production of a novel *Bacillus* strain ZBP4 on molasses was investigated. Effects of some environmental conditions on the production were also determined.

**Methods**

The bacterial growth was carried out in Nutrient Broth at 35 °C for 24 h. The medium for EPS production contained 60 g molasses, 5 g yeast extract, 5 g peptone, 1 g K<sub>2</sub>HPO<sub>4</sub>, and 0.1 g MgSO<sub>4</sub>·7H<sub>2</sub>O in one liter of distilled water. Effects of temperature (30 to 45 °C), pH (4.0- to 9.0), substrate concentration (10, 20, 30, 40, and 60 g/L molasses), nitrogen sources (ammonium sulfate, peptone, tryptone, and yeast extract), and aeration (80, 100, 150, and 200 rpm) on the EPS production were determined. For the isolation of EPS, cells were removed by centrifugation and supernatant was boiled and proteins were precipitated with trichloroacetic acid. Protein free supernatant was mixed with cold ethanol and centrifuged to precipitate EPS. Amount of EPS was determined with phenol-sulfuric acid method using glucose as standard.

**Conclusions**

The strain was produced highest amount of EPS at 60 g/L molasses concentration, 33 °C, pH 5.0. Tryptone was the best nitrogen source for EPS production. At 100 rpm agitation speed EPS production was the highest.

**FEMS-1940**

**Biotechnology and industrial microbiology**

**ASSEMBLY AND GRAFTING OF PATHWAYS FOR SYNTHESIS OF BERRY HIGH-VALUE PHENOLICS IN LACTOCOCCUS LACTIS.**

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**Background**

Plant phenolics demonstrate promising effects in the combat of cardiovascular disease, certain types of cancer, neurodegenerative diseases, allergies, diabetes and inflammation. Accordingly, the food industry actively searches for natural alternatives, such as anthocyanins, to replace synthetic dyes. The increasing consumers demand for plant-derived natural products with health-promoting (eg. nutraceuticals) and/or biotechnological applications (eg. food colorants) requires the implementation of innovative solutions to the large-scale production of such compounds. One such opportunity is offered by the exploitation of microbial hosts as cell factories.

**Objectives**

Our aim was to reconstruct the pathway for plant anthocyanin pelargonidin-O-glucoside production from flavanone naringenin in *Lactococcus lactis*.

**Methods**

In order to assemble a functional pathway, native genes from various plants and synthetic codon-optimized genes were cloned in *L. lactis*, and their expression was tested in this bacterium. Then, highly expressed genes were assembled in one anthocyanin production cluster in *L. lactis*.

**Conclusions**

In this study, we present the construction of an artificial gene cluster consisting of four native and codon-optimized plant genes for synthesis of anthocyanin pelargonidin-O-glucoside from naringenin in *L. lactis*.

**FEMS-1964**

**Biotechnology and industrial microbiology**

## **INHIBITORY EFFECTS OF VARIOUS ENVIRONMENTAL CONDITIONS ON METHANOGENIC CONSORTIA**

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### **Background**

In our previous work three methanogenic consortia were selected from environments with high concentration of methane with biological origin: (i) anaerobic digester biogas plant (AD), raw sewage sludge waste (RSS), cattle slurry (CS). Those consortia are highly specialized in stable and effective biogas production during mesophilic anaerobic digestion from maize silage.

### **Objectives**

The aim of this study was verification of: (i) the ability to effective production of biogas from maize silage by the selected microbial consortia under various temperature and pH conditions, and (ii) the stability of microbial consortia community under various temperature and pH conditions.

### **Methods**

Lab-scale anaerobic digestion process. Biogas yield: daily biogas production, methane content were analyzed by gas chromatography GC-MS. Physical and chemical analysis: pH, volatile fatty acids (VFA), chemical oxygen demand (COD), ammonium, total solids (TS), volatile solids (VS). Determination of total cell counts by fluorescence microscope at a magnification of 600, using the filter set WU for DAPI detection. DNA isolation and purification. PCR amplification. Denaturing gradient gel electrophoresis (DGGE) of archaeal and bacterial 16S rRNA.

### **Conclusions**

The obtained results showed that the selected consortia are able to produce biogas in broad range of environmental conditions. However, the process is running with a lower methane yield in psychrophilic and acidogenic condition. These results also demonstrated that selected consortia have broad spectrum of activity and could be used as a “biostarter” in start-up trial operation in different biogas plant technology process.

FEMS-1994

Biotechnology and industrial microbiology

**COMPARATIVE EFFICIENCY OF ADDING ETHANOL OR POTASSIUM METABISULPHITE IN FUEL ALCOHOL FERMENTATIONS CONTAMINATED WITH DEKKERA BRUXELLENSIS: EFFECT ON THE CELL NUMBER AND ETHANOL YIELD**

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**Background**

Fuel alcoholic fermentations contaminated with *Dekkera bruxellensis* result in decreased ethanol yield.

**Objectives**

The aim of this work is to compare the efficiency of two cell treatments carried out prior to the fermentation to control the growth of *D.bruxellensis* without affecting the starter yeast *Saccharomyces cerevisiae* and the ethanol yield, in pure and mixed cultures.

**Methods**

The treatments consisted in the addition of 13% (v/v) ethanol or 250mg/l potassium metabisulphite (PMB) to the acidic solution (pH2.0), for 2 hours at 30°C, under shaking, in which the cells were introduced. After treatment, the cells were washed twice with sterile water, centrifuged and inoculated in sugar cane juice for 12 hours. The cell number was estimated by plating the samples in WLN medium with and without actidione to count the CFU number of *D.bruxellensis* and *S.cerevisiae*, respectively. Alcohol production was also determined.

**Conclusions**

In the mixed culture, the addition of ethanol to the acidic solution had similar effect over *S.cerevisiae* and *D.bruxellensis*, however the cell number increased after the fermentation time only for *S.cerevisiae*. The ethanol production was reduced by 6%. When PMB was used, the ethanol production was decreased considerably (74%) although the number of *S.cerevisiae* was not affected. *D.bruxellensis* was not affected at all. The interaction between PMB and *S.cerevisiae* to inactivate the toxic effect of this substance may be the reason why the fermentation yield was affected considerably. The addition of 13% ethanol in the cell treatment was the most efficient and the least harmful. Support: FAPESP



**FEMS-2012**

**Biotechnology and industrial microbiology**

**LIGNINOLYTIC ENZYME ACTIVITIES AND CORN STRAW DELIGNIFICATION BY THE FUNGAL STRAIN CMU-196 OF PARACONIOTHYRIUM SP.**

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**Background**

Filamentous fungi degrade lignin by producing extracellular oxidative enzymes such as laccase (LAC), lignin peroxidase (LiP) and manganese peroxidase (MnP). These enzymes have diverse biotechnological applications, including straw delignification intended for biofuels production or ruminant feed. *Paraconiothyrium* is an ascomycete genus recently described whose species can produce ligninolytic enzymes; however, until now only few strains within the genus have been analyzed for this purpose.

**Objectives**

The aim of this work is to analyze the production of the extracellular LAC, LiP and MnP in the strain CMU-196 of *Paraconiothyrium* sp., and to evaluate the corn straw delignification by this strain.

**Methods**

Basal and induced enzyme activities were determined by incubating the strain CMU-196 in potato dextrose broth (PDB) at 24 °C and 128 rpm. Induced media were supplemented with ground corn straw (2% w/v), an aqueous extract of it (10% v/v) and CuSO<sub>4</sub> (150 µM). Corn straw delignification in a solid state fermentation was followed by scanning electron microscopy (SOM).

**Conclusions**

Maximum activity for MnP (83.72 U/ml) and LAC (103.64 U/ml) was shown at 5<sup>th</sup> day of incubation in PDB supplemented with ground corn straw. Maximum activity of LiP (25.66 U/ml) was observed at 6<sup>th</sup> day of incubation in basal medium. SOM images reveal an increase in delignification of vascular tissue of the corn straw inoculated with the fungus since week 4<sup>th</sup> until the end of treatment at week 12<sup>th</sup>. The strain CMU-196 produce high levels of the three extracellular ligninolytic enzymes and has potential for straw delignification.

**FEMS-2027**

**Biotechnology and industrial microbiology**

**GROWTH AND ETHANOL PRODUCTION OF DEKKERA BRUXELLENSIS AND SACCHAROMYCES CEREVISIAE STRAINS REGARDING THE TYPE AND CONCENTRATION OF THE CARBON SOURCE UNDER VARYING SHAKING CONDITIONS**

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**Background**

The contamination of fuel alcohol fermentations with *Dekkera bruxellensis* brings about decrease in ethanol productivity and yield.

**Objectives**

Growth and fermentation profiles of two strains of *D.bruxellensis* and one strain of *S.cerevisiae* were evaluated under varying conditions of shaking in synthetic medium with glucose or sucrose as carbon source in different concentrations. We aimed to verify if the conditions to obtain higher alcohol production are similar for both yeast species.

**Methods**

Erlenmeyer flasks containing yeast cells in 200 ml of synthetic medium containing glucose or sucrose (50, 100 and 150g/l) were maintained at 30°C for 96 h under 0, 150 or 250 rpm. Growth was monitored by optical density at 600 nm and alcohol production was determined by density.

**Conclusions**

The strains of *D.bruxellensis* displayed similar results concerning growth: it was higher under shaking conditions regardless the carbon source type and concentration and was higher in sucrose than in glucose. The best conditions to achieve higher alcohol production were not similar and it was dependent on the shaking and the carbon source concentration. For *S.cerevisiae*, the growth was higher with shaking and no effect of the carbon source type and concentration was detected. Alcohol

production was substantially higher in static cultures regardless the carbon source. Sucrose at 150g/L, which is the basic fermentation medium in static cultures, favors alcohol production by *S.cerevisiae* but it does not for *D.bruxellensis*, whose growth is stimulated by sucrose. These results confirm the role of this yeast as contaminant and shows the variability exhibited among strains. Support: FAPESP

**FEMS-2031**

**Biotechnology and industrial microbiology**

**RHODOBACTER CAPSULATUS – AN ALTERNATIVE HOST FOR THE PRODUCTION OF HETEROLOGOUS MEMBRANE PROTEINS**

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**Background**

Membrane proteins (MPs) play a crucial role in cell biology of any living organism. Nearly 30% of all open reading frames encode MPs and more than 50% of currently available pharmaceuticals target MPs. Despite their importance, the function of most MPs has not been assigned, yet.

**Objectives**

Since production of MPs is commonly limited by their hydrophobic nature, it is inevitable to generate novel expression systems that allow the high-level production of this class of enzymes. Here, we present an alternative expression system based on the bacterium *Rhodobacter capsulatus*, which is – due to its phototrophic nature – especially suited for the synthesis of MPs.

**Methods**

The facultative anaerobic purple bacterium *R. capsulatus* is able to grow either chemotrophically or phototrophically. Under latter conditions, it forms an intra-cytoplasmic membrane system (ICM) and vesicles, harboring the proteins of the host's photosystem and heterologously produced MPs. To characterize the expression properties of this system, several genes encoding monooxygenases, diagnostic-relevant MPs as well as GPCRs were cloned into a comprehensive set of novel broad host range expression plasmids of the pRho series. Subsequent accumulation, localization and activity studies were conducted to evaluate the alternative expression system.

**Conclusions**

With *R. capsulatus* and the pRho plasmids we have developed an alternative, powerful expression system for the synthesis of heterologous MPs. Our system is particularly suited for the production of monooxygenases, diagnostic-relevant proteins and GPCR-(like) proteins with yields of more than 10 mg per liter culture.

**FEMS-2040**

**Biotechnology and industrial microbiology**

**INNOVATION IN THE CONJUGATION PROCESS OF THE CAPSULAR POLYSACCHARIDE PRODUCED BY HAEMOPHILUS INFLUENZAE TYPE B LINKED TO TETANUS TOXOID**

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**Background**

*Haemophilus influenzae* type b is a Gram-negative bacterium causing pneumonia and meningitis. Its capsular polysaccharide - PRP is the main virulence factor. Vaccines based on PRP conjugated to protein are very efficient. The low yield of the conjugate has motivated the development of conjugation process to attend the demand of low-income countries. DMT-MM (4-[4,6-dimethoxy-1,3,5-triazin-2-yl]-4-methylmorpholinium chloride) used as activation molecule is an alternative to improve the conjugation reaction and the anti-PRP IgG level was statistically similar to commercial vaccine.

**Objectives**

To improve the conjugation process to make feasible the production of conjugate vaccine in large scale (g).

**Methods**

PRP (2.3g at 10g/L) was oxidized by  $\text{NaIO}_4$ , quenched with glycerol and purified by tangential ultrafiltration resulting PRP-Oxi. PRP-Oxi (1.9g at 8g/L) was mixed with adipic acid dihydrazide (ADH) followed by addition of  $\text{NaBH}_3\text{CN}$ . After 4 hours,  $\text{NaBH}_4$  was added and submitted to ultrafiltration resulting PRP-AH. PRP-AH (15g/L) was reacted with TT (15g/L) in the presence of DMT-MM (0.1M) and the conjugate product was purified by size exclusion chromatography.

**Conclusions**

In large scale some adjustment were required in order to achieve the desired yield. For instance, glycerol amount was increased to stop the oxidation (83%). In the next step the amount of ADH was raised to avoid intermolecular reaction with 94% of PRP-AH. These yields are in agreement with those obtained in small scale (mg). The last step (mg scale) yielded 46% and now we are working on gram scale. Further studies considering immunological aspects will be performed.

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**FEMS-2061**

**Biotechnology and industrial microbiology**

## **GENOME SHUFFLING OF LACTOBACILLUS RHAMNOSUS FOR IMPROVED PRODUCTION OF LACTIC ACID**

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### **Background**

The development of bio-based production leads to an increasing demand for rapid engineering of multiple complex phenotypes into a single production host. The approach of genome shuffling is providing a powerful platform for improving multiple complex phenotypes in ill-characterized hosts.

### **Objectives**

The main objectives of this study were to improve acid tolerance and lactic acid production from *Lactobacillus rhamnosus* through genome shuffling.

### **Methods**

In the present study, the genome shuffling was used to improve the acid tolerance of while simultaneously enhancing lactic acid production. A total of 10 yoghurt samples were randomly collected in sterilized glass bottles from farmers directly and were processed immediately for isolation of the lactic acid bacteria. The isolated 5 strains of *Lactobacillus rhamnosus* treated for adaptation of Low pH. Adapted wild-type strain in pH 4.0 medium was then used as the parental, or starter, for genome shuffling. All the isolated *Lactobacillus rhamnosus* strains were mutagenized using nitrosoguanidine (NTG) while genome shuffling was carried out using standard method. From the results, it was observed that, the mutants showed a small increase, from 6.1 to 11.2 g/l, in production of l-lactic acid in comparison with the Wild type strain.

### **Conclusions**

In conclusion, genome shuffling successfully improved the tolerance of *L. rhamnosus* towards acid. The research here demonstrated that genome shuffling could greatly accelerate the improvement of important phenotypes of microorganisms by developing their ability to circumvent the extreme process condition.

**FEMS-2086**

**Biotechnology and industrial microbiology**

## **MORPHOLOGY OF ASPERGILLUS TERREUS NRRL 1960 IN RELATION TO EXTERNAL MANGANESE (II) ION CONCENTRATION**

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### **Background**

Filamentous fungi in general and *Aspergilli* in particular are amongst the most important cell factories in biotechnology, as they have an intrinsic potential to produce a variety of organic acids such as citrate, gluconate and itaconate in high quantities. In order to obtain high yields of citric acid by *A. niger*, the elimination of Mn (II) ions from the medium is a prerequisite. Mycelia grown under manganese deficiency form dense and very small pellets. In this study, *A. terreus* NRRL 1960, an itaconic acid producer strain was used to study the effects of external manganese (II) ion concentration on fungal morphology.

### **Objectives**

Our primary objective was to monitor time-course changes in the morphology of *A. terreus*, grown under different external Mn<sup>2+</sup> ion concentrations.

### **Methods**

Well-controlled, strongly aerated lab-scale batch fermentations were performed. Changes in fungal morphology was monitored by image analysis. External Mn (II) ion concentrations were determined by ICP-MS.

### **Conclusions**

The overwhelmingly mycelial morphology of *A. terreus* NRRL 1960 progressively turned to pellet-like below 50 µg L<sup>-1</sup> Mn<sup>2+</sup> ion concentration. At 5 µg L<sup>-1</sup> Mn<sup>2+</sup> ion concentration and below, morphology was completely dominated by yeast-like, swollen cells.





**FEMS-2104**

**Biotechnology and industrial microbiology**

**ANTIBIOTIC RESISTANCE AND VIRULENCE PROFILE OF BACILLUS PUMILUS GROUP MEMBERS**

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**Background**

*Bacillus pumilus* present a biotechnological and pharmaceutical relevance highlighted by their ability to produce compounds with different biological activities. Moreover, they can be also found as troublesome contaminants in industrial settings and also be associated with food poisoning. Recently, phylogenetic analysis supported on different biomarkers determined the reassignment of most *B. pumilus* as *Bacillus safensis* or *Bacillus invictae*. The absence of a resistance profile to antibiotics of human and veterinary importance and also of toxin encoding genes are pre-requisites specified by EFSA when their use is intended in the food sector.

**Objectives**

To evaluate the antibiotic susceptibility, and the presence of antimicrobial resistance or entero- and emetic-toxins encoding genes in a clonally diverse *B. pumilus* group collection.

**Methods**

Minimum inhibitory concentrations to different antibiotics were determined and interpreted according to clinical breakpoints and microbiological cut-offs, in a collection comprising *B. safensis* (n=27), *B. invictae* (n=9) and *B. pumilus* (n=4). Additionally, acquired genes encoding resistance to relevant human and veterinary antibiotics (tetracyclines, macrolides, aminoglycosides, glycopeptides, phenicols and oxazolidinones) and also entero- and emetic-toxins (cytotoxin K, haemolysin, non-hemolytic and emetic toxins) were screened by PCR.

**Conclusions**

Susceptibility to all antibiotics included in interpretative criteria for their testing on *Bacillus* spp. was observed. Nevertheless, high MIC values (8-64 mg/mL) were observed for cefotaxime, a therapeutically relevant antibiotic. All *B. pumilus* group species revealed the absence of acquired antibiotic resistance and virulence genes, suggesting that there is no recognized risk of their application in the food sector or in other relevant biotechnological uses.

## FEMS-2124

### Biotechnology and industrial microbiology

#### BIOTECHNOLOGICAL POTENTIAL OF BURKHOLDERIA GLUMAE PG1

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#### Background

Microorganisms represent prolific sources for biotechnological relevant compounds which often need to be produced in heterologous hosts like *Escherichia coli*. Here, we present the Gram-negative bacterium *Burkholderia glumae* PG1 as a promising alternative production strain.

#### Objectives

*B. glumae* PG1 is known to secrete the lipase LipA which is used by the BASF SE for the production of enantiopure building blocks. A detailed genome comparison of *B. glumae* PG1 [1] with other representatives of this genus revealed that this phytopathogenic strain lacks the gene cluster encoding the biosynthesis of the important virulence factor toxoflavin.

#### Methods

Furthermore, we identified 25 clusters including PKS and NRPS for the production of secondary metabolites as well as genes encoding rhamnolipid biosynthesis enzymes resulting in the production of 20 mg/L rhamnolipids under non-optimized conditions.

We analyzed the genome of the industrial lipase production strain *B. glumae* LU8093 which was generated by random mutagenesis and identified 72 single nucleotide polymorphisms compared to the wild-type. Two of them were associated with the *lipAB* operon and affect its transcription as well as the efficiency of lipase secretion.

#### Conclusions

The identification of the putative SGNH hydrolase LipG and the construction and functional analysis of a T7 polymerase based expression strain further underline the biotechnological potential of *B. glumae* PG1.

[1] Knapp A, Voget S, Gao R, Zaburannyi N, Poehlein A, Krysciak D, Vollstedt C, Breuer M, Hauer B, Streit WR, Müller R, Daniel R, Jaeger KE. **Genome analysis and biotechnological potential of *Burkholderia glumae* PG1**. Submitted for publication.

**FEMS-2180**

**Biotechnology and industrial microbiology**

**OPTIMIZATION AND KINETICS OF THE PHYTOHORMONE PRODUCTION BY INONOTUS HISPIDUS IN STIRRED TANC REACTOR SCALE**

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**Background**

In this study, firstly, the ability of plant growth hormones (PGH) production capacity of more than a hundred Basidiomycetes isolates was screened.

**Objectives**

Plant growth regulators are commonly used by agricultural manufacturers in world wide. In our study, we have tried producing some of these plant growth regulators from a macro fungi, *Inonotus hispidus*.

**Methods**

*Inonotus hispidus* was firstly recorded as a PGH producer species and was selected for following experiments based on its 1033.65, 125.11, and 10.91 mg/L gibberellic acid (GA3), abscisic acid (ABA) and indole acetic acid (IAA) production values, respectively. Secondly, PGH producing capacity of *Inonotus hispidus* isolate was determined while the growth of the mushroom on different culture types (static, submerged and solid state). On 15th day of submerged fermentation, maximum plant growth hormones production was determined as 2478.85, 273.26, and 30.67 mg/L for GA3, ABA and IAA, respectively. Then, Plackett-Burman and response surface experimental designs were used for optimization of PGH production by *Inonotus hispidus*. Finally, the determined optimum conditions were investigated in stirred tank reactor scale to produce PGH in higher volume and reached results were, GA; 5441.54, ABA; 390.39 and IAA; 44.79 mg/L. PGH production by free and immobilized cells was also compared.

**Conclusions**

Consequently, production amounts of GA3, ABA and IAA by *Inonotus hispidus* in reactor scale were increased respectively 5.26, 3.12 and 4.1 fold according to screening step. The kinetic parameters of PGH production and growth of *Inonotus hispidus* ( $\mu$ ,  $r_s$ ,  $Q_s$ ,  $r_p$ ,  $Q_p$ ,  $Y_{p/s}$ ,  $Y_{p/x}$ ) were also determined in optimize and non-optimize conditions in Erlenmeyer flask and stirred tank reactor scales.

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**FEMS-2184**

**Biotechnology and industrial microbiology**

### **DNA APTAMERS BLOCKING ACTIVITY OF ANTHRAX LETHAL TOXIN**

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### **Background**

Anthrax is major biosecurity threat. Apart from natural outbreaks, lethality of anthrax and recalcitrance of *B. anthracis* spores to environmental factors and disinfectants made it the most feared bioterrorist weapon.

Anthrax lethal toxin (LeTx) consisting of receptor-binding (protective antigen), and protease (lethal factor) subunits is main target for development of anthrax diagnostic and therapeutic tools. Aptamers represent important platform for discovery of target-specific affinity reagents. Particularly, aptamers known to be the source of efficient allosteric protease inhibitors [1]. Immuno-aptameric PCR possesses the highest known detection sensitivity [2].

### **Objectives**

The purpose of the present study was to develop new technique aimed at rapid isolation of high-affinity aptamers suitable as diagnostic and therapeutic candidates against LeTx.

### **Methods**

In the aptamer library screening, key elements to isolate high-affinity binders are: prevention of carryover of target-specific aptamers by nonspecific oligonucleotides, and early elimination of unbound and weakly bound species. Using LeTx as the target, we developed efficient, cost and time saving protocol of enabling isolation of high-affinity aptamers by using on-rate selection, tandem affinity purification of target-specific aptamers by double proteolytic elution that leaves tightest bound aptamers associated with the eluted target, whereas the majority of binders with intermediate affinity already dissociated and removed during the competitor-based off-rate selection.

### **Conclusions**

New aptamer selection technique is useful in isolation of ultra-high affinity aptamers targeting bacterial toxins.

The work was supported by Russian scientific fund research grant № 14-15-00630.

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**FEMS-2191**

**Biotechnology and industrial microbiology**

**SELECTION OF NANNOCHLOROPSIS OCEANICA CELLS WITH HIGHER INTRACELLULAR LIPIDS CONTENT BY FLUORESCENCE ACTIVATED CELL SORTING.**

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**Background**

Microalgae are now the focus of interest due to their ability to produce a intracellular lipid feedstock that is suited to conversion in biodiesel. *Nannochloropsis oceanica* (N.O.) is a unicellular green alga intensely investigated because of the natural ability to form intracellular lipid bodies that contain triacylglycerol.

As a way to select the cells with spontaneous higher amount of intracellular lipids were separated those cells from a heterologous population using the technique of fluorescence activated cell sorting (FACS).

**Objectives**

Visualization of intracellular lipid droplets of N.O. by epifluorescence microscopy.

Visualization and selection of cells with high intracellular lipids content from heterologous populations of N.O. cultures by FACS.

Quantification of total lipids in selected cellular populations of N.O. under different culture conditions

**Methods**

The microalgal cultures were suspended in DMSO and stained with Bodipy 505/515. Flow Cytometer (BD Influx™ cell sorter) was used to detect the fluorescence signal of Bodipy. Neutral lipids stained showed a particular emission peak at 530 nm.

Total lipid fatty acid profiles were determined by GC of methyl esters.

**Conclusions**

Intracellular lipid droplets stained with Bodipy 505/515 was easily visualized by epifluorescence microscopy in N.O. cells.

The FACS technique allowed the visualization and separation of cellular populations enriched in intracellular lipids from liquid cultures of N.O.

Analysis of lipids quantification confirmed the obtaining of cellular populations with higher amounts of total lipids and fatty acids reaching 46% of total lipids by mg of dry weight.

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**FEMS-2228**

**Biotechnology and industrial microbiology**

**Fungicidal activity of exo- and endo-polysaccharides from Amazonian fungi**

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**Background:**

Endophyte fungi are usually find in Amazon Rainforest with high biodiversity, and biological and chemical potential. They can be found as endophytes, phytopathogens or saprophytes. *Candida* is a fungal pathogen responsible for opportunist infections in humans.

**Objectives:**

To produce exo- and endo-polysaccharide extracts from *Pestalotiopsis* sp. and *Talaromyces* sp., and assay them against *Candida albicans*.

**Material and Methods:**

Two fungi from the GEMMA group fungal collection were cultivated for 18 days on PDY medium.. Then the mycelium was separated from the medium. After add to culture medium from each fungus 1L of methanol, the mixture was left for 48 hours at 4 °C. The precipitate formed was separated by centrifugation and the solvent in the supernatant was evaporated in rotary evaporator. The aqueous phase was subjected to liquid-liquid extraction with ethyl acetate and the acetate extract was concentrated by rotary concentration.

**Conclusions:**

Three samples were selected for candidicidal assays: (1) *Talaromyce* inorganic partitioned, (2) *Pestalotiopsis* mycelium, and (3) *Talaromyce* culture aqueous. All extracts had revealed candidicidal activity and were subsequently tested at the concentrations of 1000, 750, 500 and 250 µg.mL<sup>-1</sup> to determine the minimum inhibitory concentrations. The sample (1) and (3) showed no satisfactory inhibition while (2) showed inhibition at 500 µg/ml.

**FEMS-2284**

**Biotechnology and industrial microbiology**

**EXPRESSION, PURIFICATION AND CHARACTERIZATION OF A BIFUNCTIONAL 99-KDA PEPTIDOGLYCAN HYDROLASE FROM *PEDIOCOCCUS ACIDILACTICI* ATCC 8042**

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**Background**

Lactic acid bacteria inhibit the growth of pathogens and food deleterious microorganisms because they produce substances with antibacterial activity, such as peptidoglycan hydrolases (PGH). *Pediococcus acidilactici* ATCC 8042 has been reported to inhibit pathogenic microorganisms such as *Staphylococcus aureus* through the production of two proteins with lytic activity, which are approximately 110 and 99 kDa. The 99-kDa PGH has high homology to a putative protein reported in *Pediococcus acidilactici* 7\_4, where two different lytics domains have been identified but not characterized.

**Objectives**

The aims of this work were cloning, expression, purification and biochemical characterization this bifunctional enzyme.

**Methods**

The 99-kDa PGH was cloned and expressed successfully and showed activity against *Micrococcus lysodeikticus*. The protein was then purified using gel filtration chromatography. For the characterization 4-Nitrophenyl-*N*-acetyl- $\beta$ -D-glucosamine was used as substrate.

**Conclusions**

The pure protein showed an optimal pH for antibacterial activity and stability of 6.0 and 5.0 to 7.0, respectively. The optimal temperature for activity was 60°C, and it lost all activity after incubation at 70°C for 1 h. The number of strains susceptible to the recombinant 99-kDa enzyme was lower to those lysed by the mixture of the 110- and 99-kDa PGHs of *P. acidilactici*, which indicates that there might be synergy and interactions between these two enzymes. This is the first peptidoglycan hydrolase from LAB that has been shown to possess two lytic sites. The results of this study will aid in the design of new antibacterial agents from natural origins that can combat foodborne disease and improve hygienic practices in industrial sector.

**FEMS-2286**

**Biotechnology and industrial microbiology**

## **BIOCHEMICAL CHARACTERIZATION OF A SPORE LACCASE FROM A HALOTOLERANT BACTERIUM, BACILLUS SP.**

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### **Background**

Laccases, one of polyphenol oxidases, have many applications in different industries and can be used in bioremediation, organic synthesis, and biosensors.

### **Objectives**

In present study, different bacterial strains were screened to find laccase producing strain. Biochemical characterization and Crystal violet decolorization effect of the spore laccase from a spore producing strain was examined.

### **Methods**

As total 50 bacterial strains isolated from a soil sample of a chromite mine in Iran were screened for laccase production on nutrient agar medium supplemented with 3% (w/v) NaCl and 0.02% (v/v) guaiacol. Spores from a spore forming Gram-positive strain showed laccase activity. The strain was identified and pH and temperature optima for the enzyme activity were determined. Effects of inhibitors and metal ions on the enzyme activity were studied. Decolorization of Crystal violet was investigated by spore laccase.

### **Conclusions**

The closest relative of the laccase producing strain was *Bacillus safensis* FO-036b<sup>(T)</sup>. The spore laccase showed optimal activity at pH 5 and 35°C, had a good tolerance to metal ions, and retained 104% of its initial activity against 1 mM of NaN<sub>3</sub>. The enzyme decolorized 64% of 10 mg/L Crystal violet, so it could be a good candidate to bioremediation applications.

**FEMS-2339**

**Biotechnology and industrial microbiology**

### **TO LOW COST FERMENTATION PROCESSES**

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### **Background**

Fermentation processes have been used for over 45 centuries, especially for the production of food, with ethanol and lactic acid as the main biogenic compound. These products have a high added value, because they are used as stimulant and/or because of their food preserving nature. This high added value allows significant costs for the fermentation process.

During the last 30 years fermentation technology has also been applied for the production of chemicals and fuels. These products have to compete with low-cost petrochemicals, putting restrictions on the costs of the fermentation processes. The current state-of-art fermentation technology is often too expensive for competitive production of chemicals and fuels.

In the same period the instruments to modify microorganisms have evolved considerably. The ability to build whole genomes, the application of –omic techniques and genome scale modelling give unprecedented possibilities to design microorganisms for optimal product formation.

### **Objectives**

Instead of adapting the process to the microorganisms to reach the best results, it is now time to develop strategies which adapt the microorganisms to the process.

### **Methods**

Key drivers for process improvement are the reduction of oxygen requirement by redox engineering, the increase of product tolerance and the separation of the product from the aqueous broth by phase separation.

### **Conclusions**

In this paper/presentation we describe our activities to realize this and estimate the cost reduction that can be realized. Itaconic acid and fatty acids will be shown as examples. A cost reduction to a value below that of contemporary ethanol production (800 €/kg) seems feasible.

**FEMS-2347**

**Biotechnology and industrial microbiology**

## **REACTIVE OXYGEN SPECIES REGULATE LOVASTATIN BIOSYNTHESIS IN *ASPERGILLUS TERREUS***

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### **Background**

Lovastatin (LOV) is a secondary metabolite, produced by *Aspergillus terreus*. It has great commercial importance since it lowers cholesterol levels in blood. Studies from our group, showed a link between reactive oxygen species (ROS) and LOV biosynthesis in submerged (SmF) and solid-state fermentation (SSF). Our results showed that *sod1* gene (oxidative stress defense enzyme) was intensely expressed during rapid growth phase (or trophophase) in LOV fermentations, but it was down regulated in production phase or idiophase. Probably because of this, in that moment ROS levels increased, generating an oxidative state during idiophase (Miranda et al 2013).

### **Objectives**

The objective of the present work was finding evidence that ROS contribute to the regulation of lovastatin biosynthesis.

### **Methods**

ROS were manipulated during LOV fermentations. Exogenous antioxidants were used to eliminate or reduce ROS accumulation during lovastatin fermentations. Its effect on lovastatin production was determined. Also, its effect on the expression of LOV genes (*lovE* and *lovF*), and on genes *yap1* and *srrA* (encoding oxidative stress defense transcription factors) was determined by Northern analysis.

Similar (complementary) experiments, increasing ROS by H<sub>2</sub>O<sub>2</sub> addition to LOV fermentations, were also performed.

### **Conclusions**

1. ROS accumulation in idiophase is not only necessary for the normal production of lovastatin, but contributes to the transcriptional regulation of the biosynthetic genes, including the specific regulatory gene *lovE*.



2. ROS regulation could be mediated by oxidative-stress-response transcription factors: Yap1 might be acting as a negative regulator; while SrrA could play an important role in positively controlling lovastatin production.

**FEMS-2348**

**Biotechnology and industrial microbiology**

**IDENTIFICATION AND BIOACTIVE POTENTIAL OF ENDOPHYTIC FUNGI  
ISOLATED FROM MEDICINAL PLANT WITHANIA SOMNIFERA**

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**Background**

Endophytes are microbes that colonize living, internal tissues of plant without causing any immediate or negative effect. Various studies have shown that endophytes are endowed with great potential for human welfare like having potential in the form of antibiotics, antiviral, anti asthmatic, antioxidant and anticancer compounds. Endophytes from medicinal plants have become a hot topic for metabolite discovery. There is still a lot of potential of endophytes which need to be discovered for pharmaceutical applications. Keeping these things in consideration, the aim of the present study was to isolate fungal endophytes from different accessions of medicinal plant *Withania somnifera* and to screen the endophytes for the production of various bioactive molecules.

**Objectives**

To isolate the endophytic microorganisms from *Withania somnifera* (Ashwagandha) & screen for antimicrobial activity against a wide range of pathogenic microorganisms

**Methods**

Isolation of endophytes using the standard methodology published earlier

**Conclusions**

Out of 7 endophytes, four isolates were identified by ITS amplification and sequencing. All the four isolates showed significant similarity with *Alternaria alternata*, *Alternaria tenuissima* strain, *Alternaria* sp. and *Alternaria compacta*.

The isolated endophytes were also screened for antimicrobial activity against a wide range of pathogenic microorganisms and after analyzing the results some of the endophytes showed promising antimicrobial activities.

Our results confirm that the isolated endophytes could serve as an alternative source of various bioactive molecules of novel structure and function as endophytes are still largely unexplored.

**FEMS-2370**

**Biotechnology and industrial microbiology**

**SECRETION AND FUNCTIONAL DISPLAY OF FUSION PROTEINS THROUGH THE CURLI BIOGENESIS PATHWAY**

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**Background**

Curli are functional amyloids expressed as fibres on the surface of Enterobacteriaceae. Contrary to the protein misfolding events associated with pathogenic amyloidosis, curli are the result of a dedicated biosynthetic pathway. A specialized transporter in the outer membrane, CsgG, operates in conjunction with the two accessory proteins CsgE and CsgF to secrete curlin subunits to the extracellular surface, where they nucleate into cross-beta strand fibres.

**Objectives**

Here we investigate the substrate tolerance of the CsgG transporter and the capability of heterologous sequences to be built into curli fibres.

**Methods**

We genetically fused different proteins to the C-terminus of csgA.

**Conclusions**

Non-native polypeptides ranging up to at least 260 residues were exported when fused to the curli subunit CsgA. Secretion efficiency depended on the folding properties of the passenger sequences, with substrates exceeding an approximately 2 nm transverse diameter blocking passage through the transport channel. Secretion of smaller passengers was compatible with prior DsbA-mediated disulphide bridge formation in the fusion partner, indicating that CsgG is capable of translocating non-linear polypeptide stretches. Using fusions we further demonstrate the exported or secreted heterologous passenger proteins can attain their native, active fold, establishing curli biogenesis pathway as a platform for the secretion and surface display of small heterologous proteins.

**FEMS-2379**

**Biotechnology and industrial microbiology**

**STUDY OF THE BIOTECHNOLOGICAL POTENTIAL OF EXTREMOPHILE ACTINOMYCETES FOR THE BIOCONTROL OF FUNGAL DISEASES OF CEREAL CROPS UNDER VARIOUS ENVIRONMENTAL CONDITIONS**

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**Background**

In the recent years, the applicability of actinomycete strains as probiotics for plants, serving as agents of biological control of plant diseases, has been actively investigated.

**Objectives**

415 strains of extremophile actinomycete strains from the unusual ecosystems of Kazakhstan.

**Methods**

Antifungal properties of extremophilic actinomycetes were studied against the fungal pathogens of grain cultures (genera *Fusarium* and *Aspergillus*) by agar diffusion method.

**Conclusions**

104 actinomycetes strains from the extreme ecosystems of Kazakhstan (solonchak, takirs and takir-type soils, solonetz and solod) showed antifungal activity against the fungal pathogens of grain cultures (*Fusarium sp.*, *Aspergillus niger*). 20 strains of extremophilic actinomycetes have been selected, capable of growing at a low content of nutrients in the medium, in the presence of high salt concentrations ( $\geq 5\%$ ) and pH level ( $\geq 9.0$ ). They have the ability to inhibit the growth of phytopathogenic fungi (diameter of inhibition zone 20-30 mm) in the different environmental conditions - neutral, in the presence of high NaCl concentrations (5% or more), and high pH values (9-11). This practically valuable feature is especially important for the plant cultivation since many biopreparations developed for neutral ecological niches become useless after salinization or alkalization of the soil. Scientific novelty of the research results lies in the use of biotechnological potential of extremophilic actinomycetes in the development of biological preparations for plant cultivation under various environmental conditions.



**FEMS-2400**

**Biotechnology and industrial microbiology**

## **THERMOPHILIC FUNGI FOR EFFICIENT PLANT BIOMASS DEGRADATION**

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### **Background**

A major challenge in the sustainable production of biofuels and biochemicals is efficient enzymatic conversion of plant biomass into monomeric sugars. Most enzyme mixtures are currently produced by a small selection of fungal species (e.g. *Trichoderma reesei*, *Aspergillus niger*). However, the fungal kingdom holds many more fungal species which produce enzyme mixtures with beneficial characteristics such as high (hemi-)cellulase activity and high thermostability. *Sordariales* is one of the few fungal orders with thermophilic isolates, of which many have been associated with the production of thermostable enzymes.

### **Objectives**

The aim of our study is to assess the diversity within *Sordariales* for efficient plant biomass degradation.

### **Methods**

Phylogenetic analysis and growth analysis revealed that optimal growth temperature is a polyphyletic trait within *Sordariales* with separate mesophilic, thermotolerant and thermophilic clades. Four thermophilic clades were clearly distinguished: *Myceliophthora* species, *Thielavia terrestris*, *Chaetomium thermophilum*, and *Mycothermus thermophilus*. Thermophiles within *Myceliophthora* showed the most potential as efficient plant biomass degraders. Especially *M. heterothallica* had good growth on a large range of substrate and was able to produce offspring with a large physiological and genetic variety. Crossing and selection strategies were used to further improve *M. heterothallica* in degrading biomasses such as sugarbeet pulp and spruce. The mechanisms behind improved biomass saccharification were understood by proteomics and genome analysis.

### **Conclusions**

This study showed the strategic strength of combining fungal diversity with specific selection strategies to find enzyme mixtures with interesting industrial properties.

**FEMS-2419**

**Biotechnology and industrial microbiology**

## **PREPARATION OF ACIDIC AMINOPHOSPHONATES USING FUNGI**

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### **Background**

Biotransformation is substrate transformation to desired product by using suitable kind of biocatalysts. Nowadays, it is an alternative tool for the development sustainable technologies for the production of chemicals and drugs. Aminophosphonates are analogues of amino acids in which a carboxylic moiety is replaced by phosphonic functionality or related groups. They are compounds with stable carbon to phosphorus bond and have wide range of biological activities and variety of applications in industry [Żymańczyk-Duda, *Phosphorus, Sulfur and Silicon*, **2008**, 183; Mucha A., *J. Med.Chem.* **2011**, 54]. Biosynthesis of chiral, organophosphorous compounds is still not fully explored field of science.

### **Objectives**

The aim of work was selection of the whole-cell biocatalysts with hydrolytic activity for preparation of phosphonate analogues of aspartic acid. Thus, biocatalytic methods, which allowed obtaining derivatives of aminophosphonic acids *via* biohydrolysis of lactam ring were elaborated.

### **Methods**

To obtain phosphonic analogue of aspartic acid *via* biohydrolysis of O,O-dimethyl-4-oxoazetidin-2-ylphosphonate, whole cells of *P. minioluteum* were used as a biocatalyst. NMR spectra interpretation allowed postulating possible mechanism of presented microbial conversion as amide bond hydrolysis. Biohydrolysis was only achieved in deionized water as bioconversion medium. Application of another reaction media caused partial splitting of the substrate during biotransformation process. The separation process required reverse phase chromatography to obtain product from biotransformation mixture.

### **Conclusions**

Presented method is simple and quite efficient. Application of *P.minioluteum* as a biocatalyst resulted in production of desired product with moderate yield. It means, that process is a good starting point for further scaling up the process.





**FEMS-2449**

**Biotechnology and industrial microbiology**

**SELECT ANTIMICROBIAL PEPTIDES (AMPS) AS BACTERICIDAL AGENTS FOR EX VIVO STORED HUMAN PLATELETS**

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**Background**

Bacterial contamination of *ex vivo* stored human platelets and the transmittance of sepsis by platelet transfusion represent the highest infectious disease risk in transfusion medicine today.

**Objectives**

An alternative approach to detection of contaminated units is pathogen reduction technology that directly inactivates or prevents the replication of pathogens present in platelet products. We have developed a novel approach to pathogen reduction, one that promises to be more specific and less damaging to the platelet product and at the same time effective against 6 bacterial species common to transfusion of platelets.

**Methods**

The methodology is based on treatment of platelets with small synthetic peptides named PD1-PD4 derived from the thrombin-induced human platelet-associated antimicrobial proteins, and repeats of Arg-Trp (RW1-RW5), and their selected combinations. This treatment demonstrated microbicidal activity against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Bacillus cereus* in the spiked platelet samples. We also demonstrated that AMP treated platelets maintain their *in vitro* properties during 7 days of storage. Preclinical evaluation of peptides PD3, PD4, and RW2-RW5 demonstrated that these AMPs do not adversely affect the human platelet recovery and survival in a SCID mouse model. We have further shown that the peptides are not immunogenic in rabbits, and do not affect platelet performance in *in vitro* tests.

**Conclusions**

The body of work presented here provides a proof of a concept that AMPs could be incorporated into a novel platelet-sparing pathogen reduction treatment and warrants further study.

**FEMS-2467**

**Biotechnology and industrial microbiology**

**STABLE VECTORS FOR EFFICIENT GENE EXPRESSION IN RALSTONIA  
EUTROPHA H16**

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**Background**

The Gram-negative  $\beta$ -proteobacterium *Ralstonia eutropha* H16 is primarily known for polyhydroxybutyrate (PHB) production and its ability to grow chemolithoautotrophically by using CO<sub>2</sub> and H<sub>2</sub> as sole carbon and energy sources. *R. eutropha* H16 has also attracted significant interest for its ability to metabolize heavy metals, to degrade a variety of chloroaromatic compounds or chemically related pollutants.

**Objectives**

The use of *R. eutropha* H16 as a production organism under chemolithoautotrophic growth conditions is favored. However, inducible expression systems are yet required for gene expression in *R. eutropha* H16. The aim of this work was to identify inducible expression systems for the use in *R. eutropha* H16 facilitating gene expression.

**Methods**

A family of efficient and highly stable plasmid expression vectors was created for the use in *R. eutropha* H16 by applying various biosynthetic methods.

**Conclusions**

The use of promoters derived from bacteriophage T5 was described in this work and hereby the J5 promoter proved to be the strongest promoter yet to be applied in *R. eutropha* H16. Moreover, the implementation of the RP4 partition sequence in plasmid design increased plasmid stability significantly and enables fermentations with marginal plasmid loss of recombinant *R. eutropha* H16 for at least 96 h. The utility of the new vector family in *R. eutropha* H16 is demonstrated by providing expression data with different model proteins and consequently further raise the value of this organism as cell factory for biotechnological applications including protein and metabolite production.

**FEMS-2509**

**Biotechnology and industrial microbiology**

## **SCREENING FOR CELLULOLYTIC ACTIVE NOVEL ANAEROBIC FUNGI**

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### **Background**

Lignocellulosic plant material is degraded by anaerobic fungi (AF) with their rhizoid and various free and cellulosome-bound lignocellulolytic enzymes. AF could thus be used to improve biogas production from fiber-rich residues. AF were isolated from faeces of animals living on a high fiber diet. Screening them for the cellulolytic most active AF with conventional tests are labor intensive and time consuming, a quantitative Real-Time PCR (qPCR) routine for glycosyl hydrolase family 5 (GH5) endoglucanase should be developed as a suitable alternative.

### **Objectives**

New anaerobic fungal isolates should be checked and compared for their cellulolytic potential by parallel determination of GH5 mRNA production and conventional parameters. Thus anaerobic fungi with outstanding cellulolytic abilities shall be identified.

### **Methods**

To identify the best moment for mRNA extraction, growth curves of the isolates were recorded, and mRNA was extracted at several points. Additionally gas pressure was measured to see if mRNA production, growth and gas pressure developed accordingly. Reverse-transcribed cDNA was quantified in qPCR with primer system AF Endo designed to specifically detect GH5 endoglucanase of AF.

### **Conclusions**

Novel AF isolates were achieved from a Cameroon sheep, a Kiang, an alpine goat and a domestic yak. In experiments carried out with two of the isolates the GH5 RT-qPCR system tested successfully. GH5 mRNA production was maximal after 18h with similarly developing growth, gas pressure and mRNA level. Further AF species are being tested for their cellulolytic activity. A future study shall compare gained results with the real life cellulolytic ability of tested AF.



**FEMS-2519**

**Biotechnology and industrial microbiology**

**INVESTIGATION OF SYNTHETIC LIGNIN BIOMODIFICATION BY PHANEROCHAETE CHRYSOSPORIUM : ANALYSIS OF STRUCTURAL CHANGES OF SYNTHETIC LIGNIN AND EFFECT OF MICROBIAL ENZYMES**

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**Background**

These days, there has been a great interest in lignin as one potential renewable source for conversion into chemicals and materials. Lignin is complex and amorphous polymers, therefore, it needs to be modified to meet properties required for industrial uses.

White rot fungi secreting ligninolytic enzymes are promising for enzymatic modification, because they can degrade lignin selectively and more rapidly than other micorbes. Therefore, comprehending of biomodification process by white rot fungi is necessary for industrial application of microbial treated lignin.

**Objectives**

The biomodification of synthetic lignin by white rot fungus, *Phanerochaete chrysosporium*, was investigated in this study. The fungal secretomes and structural changes of the fungal treated synthetic lignin were examined.

**Methods**

Synthetic lignin used in this study was synthesized from coniferyl and sinapyl alcohol. This polymer, dehydrogenative polymer (DHP) was used as substrate to elucidate the mechanism of microbial modification of lignin.

Detailed structural analysis of lignin were conducted as follows : phenolic hydroxyl group of DHP was analyzed by aminolysis reaction, and the molecular weight and changes of ether bonds in DHP was analyzed by gel permeation chromatography and nitrobenzene oxidation method, respectively. For examining the effects of fungal secretomes in biomodification of synthetic lignin, protein contents by using protein chip and activities of ligninolytic enzymes were measured, respectively.

**Conclusions**

Consequently, this study is expected to help to understand the biomodification mechanism of synthetic lignin by *P. chrysosporium*, and to evaluate availability of *P. chrysosporium* in biomodification of lignin for value added application of lignin.

**FEMS-2531**

**Biotechnology and industrial microbiology**

**HOMOLOGOUS AND HETEROLOGOUS EXPRESSION OF DEHYDROGENASES AND OXIDOREDUCTASES OF RALSTONIA EUTROPHA H16**

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**Background**

*Ralstonia eutropha* is a Gram-negative, strictly respiratory facultative chemolithoautotrophic bacterium which can use H<sub>2</sub> and CO<sub>2</sub> as sole sources of energy and carbon in the absence of organic substrates. It has attracted great interest for its ability to degrade a large list of chloroaromatic compounds and chemically related pollutants. Furthermore it was already applied for the production of biodegradable polymer polyhydroxyalkanoates on an industrial scale. *R.eutropha* serves as a model organism for the mechanisms involved in the control of autotrophic carbon dioxide fixation, hydrogen oxidation and denitrification.

**Objectives**

We are interested in establishing specialized *R.eutropha* based cell factories by genetic engineering. The particular interest is constructing cells efficiently performing oxidoreductase reactions by overexpression of homologous and/or heterologous enzymes. One of the main types of oxidoreductase reactions is performed by dehydrogenases, which have a wide range of possible biotechnological applications.

**Methods**

A selection of alcohol dehydrogenases as well as short chain dehydrogenases of *R.eutropha* H16 was cloned and expressed in native versions in *E.coli* as well as in *R.eutropha* H16 in order to study basic functional capability of these enzymes. For each of the enzymes cofactor specificity and the substrate range, both for oxidation and reduction way was analysed in a photometric assay by the detection of NAD and thereby specific activity units were calculated.

**Conclusions**

Two of the tested enzymes showed high selectivity toward s-enantiomers for all tested secondary alcohols with NADP<sup>+</sup> as preferable cofactor. Furthermore a difference in enzyme activity depending on the expression host could be observed.

**FEMS-2532**

**Biotechnology and industrial microbiology**

## **METHANOTROPH-CATALYZED BIOCONVERSION OF METHANE TO METHANOL**

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### **Background**

Methane has attracted much attention as the alternative chemical feedstock and conversion of methane to methanol has attracted enormous interest. Indirect chemical conversion of methane to methanol is associated with a number of problems including low energy efficiency and high capital cost.

### **Objectives**

We investigated a batch methane-to-methanol conversion in high yield using *Methylosinus trichosporium* OB3b whole cells. Cultivation and conversion relevant parameters including air/methane ratio and copper concentration were optimized.

### **Methods**

*M. trichosporium* OB3b was cultured in a 600 mL joint flask with rubber septum containing 200 mL NMS medium with supply of 7:3 air/methane ratio at 30 °C. Various chemical inhibitors for methanol dehydrogenase were screened and added to accumulate methanol. Sodium formate was added as a reducing power source to maintain methane monooxygenase activity.

### **Conclusions**

In the presence of 100 mM potassium phosphate as MDH inhibitor, 0.4 g/L methanol was accumulated and more than 70% (mol methanol/mol methane) of methane was converted to methanol. Volumetric productivity of 0.049g/L/h was obtained in the batch reaction with *M. trichosporium* OB3b as the biocatalyst. Biocatalytic conversion using methanotrophic microorganism will offer an energy-efficient method in commercialization of methane-to-methanol.

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**FEMS-2535**

**Biotechnology and industrial microbiology**

**OPTIMIZATION OF CULTURE CONDITIONS FOR L-ASPARAGINASE  
PRODUCTION BY HALOPHILIC BACTERIUM, VIBRIO SP STRAIN GBFX3**

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**Background**

Increased amino acid requirement of malignant cells is exploited in metabolic antitumor therapy, e.g., enzymotherapies based on asparagine or glutamine deprivation. L-Asparaginase (L-Asparagine amidohydrolase; EC 3.5.1.1) which catalyzes the conversion of L-Asparagine to aspartic acid and ammonia is one of promising enzyme in pharmaceutical industries. A wide variety of microorganisms have L-asparaginase. Halophilic bacteria may contain L-asparaginase with novel immunological properties that can be used in hypersensitive patients because of modified surface structures.

**Objectives**

The main objective of this study was to optimize the culture conditions for L-asparaginase production by selected halophilic strain.

**Methods**

The effect of different concentrations of NaCl and glucose ranging from 0-10% and 0-1% respectively and the influence of different carbon sources such as saccharose, maltose, glucose and fructose on the enzyme production was evaluated by varying one factor at a time. The effect of pH (5–9) and temperature (25-35-45 °C) were also studied. After 72h incubation, the samples were collected by centrifugation for the determination of L-asparaginase activity by the nesslerization method.

**Conclusions**

A halophilic bacterium *Vibrio* sp. Strain GBFx3 which was isolated from Gomishan Lake at Golestan province of Iran was screened for L-asparaginase activity. The highest amount of L-asparaginase production was observed in 0.5% glucose or 0.5% saccharose as sole carbon source, 2.5% NaCl, 34°C and pH 8 which were 0.63, 0.5, 0.4 and 0.43 IU/ml respectively.

**FEMS-2546**

**Biotechnology and industrial microbiology**

**DEVELOPMENT OF A POLYFUNCTIONING PROLONGED-ACTION BIOLOGICAL PREPARATION FOR CROP PRODUCTION BASED ON THE RHIZOSPHERE BACTERIAL STRAINS WITH ANTIFUNGAL AND PLANT-GROWTH STIMULATING PROPERTIES**

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**Background**

Associations of the rhizosphere bacterial strains belonging to the genera *Pseudomonas* and *Azotobacter* simultaneously possess both antifungal and plant-growth stimulating properties and are the basis for new trends in the development of biological preparations for crop production.

**Objectives**

Strains *Pseudomonas* R-7 and *Azotobacter* A-6 isolated from the rhizosphere of tomato crops cultivated in Kazakhstan.

**Methods**

Antagonistic properties of the bacteria were studied using agar diffusion method against the pathogens of fungal diseases in tomatoes of the genera *Phytophthora*, *Fusarium*, *Alternaria*, *Cladosporium*. Growth-stimulating properties of bacteria were studied by inoculation of tomato seeds with a culture liquid of bacteria with a cell titer of  $n \times 10^9$ .

**Conclusions**

Strain *Pseudomonas* R-7 showed antagonism against phytopathogenic fungi: *Phytophthora* sp. - 19 mm, *Fusarium* sp. - 16 mm, *Alternaria* sp. - 23 mm, *Cladosporium* sp. - 18 mm. When using a strain *Azotobacter* A-6, the growth inhibition zone for *Phytophthora* sp. was 22 mm, *Fusarium* sp. - 24 mm, *Alternaria* sp. - 23 mm, *Cladosporium* sp. - 20 mm. Strain *Azotobacter* A-6 also enhanced the tomato germination capacity by 22.0% as compared with the control, increased the plant height by 15.5%, root length by 26.3%, plant mass by 15.0 %, and number of leaves by 33.3%.

A composition of the new biological preparation for crop production has been formulated based on the strains of rhizosphere bacteria *Pseudomonas* P-7 and *Azotobacter* A-6, compatibility of the strains under cultivation and storage examined, optimal ratio of bacterial microorganisms and soil extract in the combined culturing selected, ensuring the continued viability of cells within a scheduled storage period.



**FEMS-2616**

**Biotechnology and industrial microbiology**

**CLONING AND OVEREXPRESSION OF A NEW LIP GENE FROM  
STENOTROPHOMONAS MALTOPHILIA PSI-1 - PURIFICATION AND PARTIAL  
CHARACTERIZATION OF THE RECOMBINANT LIPASE**

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**Background**

Lipases catalyze esterification, interesterification and transesterification reactions and have gained serious attention for the production of sustainable biofuels, motivated by environmental concerns combined with the depletion of fossil fuels.

**Objectives**

The aim of this study was the isolation of new strains bearing high lipase activity in order to exploit their lipases in biofuel production.

**Methods**

42 environmental strains, isolated from sludge of a waste treatment facility (Psittaleia, Greece), were screened for the presence of lipase activity, using a rhodamine B procedure. One isolate, identified as *Stenotrophomonas maltophilia* (named Psi-1) by means of IGS-based molecular taxonomy, was selected for further analysis. A 1.203 bp amplicon of Psi-1 corresponding to a putative secreted lipase gene (as revealed by bioinformatic analysis) was cloned and overexpressed in a pET29c - *E. coli* BL21[DE3] system. The recombinant protein was purified using Ni-NTA chromatography. Lipase activity was verified by in-gel assays (zymograms) and photometric biochemical assays.

**Conclusions**

A novel lipase gene from a new *Stenotrophomonas maltophilia* isolate was identified, cloned and overexpressed. The recombinant enzyme was proved to exhibit lipase activity and has a potential as a catalyst for biofuel production.

**Acknowledgments**

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**FEMS-2620**

**Biotechnology and industrial microbiology**

**BIOCATALYZED KINETIC RESOLUTION OF CHIRAL DERIVATIZING AGENT: 2-BUTYRYLOXY-2-(ETHOXY-P-PHENYLPHOSPHINYL)ACETIC ACID WITH IMMOBILIZED WHOLE-CELL SYSTEM.**

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**Background**

The use of enzymes as biocatalysts for the preparation of optically pure compounds is becoming a common method especially in the pharmaceutical industry [Nestl B.M., 2011, *Curr Opin Chem Biol*]. Obtaining enantiopure active compounds with a defined configuration appeared to be more desirable, not only because of effectiveness of such pharmaceuticals, but also because of safety requirements [Ogawa J., 2002, *Curr Opin Biotech*]. Immobilized whole-cells are one of the biocatalyst form that can be used in biotransformation processes. This system allows reusing biocatalyst, facilitates downstream processes and enhance the stability of the enzyme. Moreover, use of immobilized catalyst in flow reactor allows carrying out continuous processes.

**Objectives**

The aim of the study was the kinetic resolution of a racemic mixture of 2-butyryloxy-2-(ethoxy-P-phenylphosphinyl)acetic acid by biotransformation using immobilized whole-cell biocatalyst. *Penicillium oxalicum* has been reported as an active biocatalyst in the hydrolysis of tested compound in shake flask system [Serafin M., 2014, *Chemik*]. Optically pure products can find application as chiral discriminator and building block of defined absolute configuration in synthesis of biologically active compounds such as pharmaceuticals or pesticides.

**Methods**

The activity of biocatalyst, cells immobilized in two ways, was tested toward racemic mixture of starting compound. Products were analyzed by nuclear magnetic resonance spectroscopy <sup>31</sup>P NMR with the addition of quinine as a chiral solvating agent.

**Conclusions**

Simple and effective method of whole-cell immobilization was elaborated. Activity of biocatalyst was confirmed by hydrolysis of tested compound with 50% of conversion



degree and high enantiomeric excess. Developed system allowed to enlarged the scale of the process.

**FEMS-2629**

**Biotechnology and industrial microbiology**

## **ITACONIC ACID PRODUCTION IN ASPERGILLUS NIGER**

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### **Background**

The filamentous fungus *Aspergillus niger* has a long tradition of safe use in industry in the production of enzymes and organic acids. Furthermore, it can grow on a wide range of substrates under various environmental conditions and has an optimized pathway towards producing organic acids, as indicated by its high production titers of citric acid in submerged fermentations (>300 g/L). These features make it an interesting host for the production of itaconic acid (IA), a high-value building block chemical with a broad application potential in the chemical industry. We have successfully identified the IA production pathway of *Aspergillus terreus* and engineered it into *A. niger*. The resulting *A. niger* strain is able to produce IA at low levels.

### **Objectives**

In order to increase the yield we have tested various medium compositions and fermentation conditions. This resulted in an optimization of the fermentation medium and cultivation conditions, accompanied with an enhancement of yield and titer of IA production. Moreover, recently we have discovered a novel pathway found, to our knowledge, exclusively in *A. niger* that enables higher productivity, titer and yield of IA. This novel pathway provides a platform for further improvement of organic acid production in *A. niger*.

### **Methods**

Fungal transformation and fermentation

### **Conclusions**

This novel pathway enabled increased IA acid productivity, yield and titer.

**FEMS-2632**

**Biotechnology and industrial microbiology**

**COMPARATIVE GENOMICS APPROACH FOR BUILDING A COMPLETE AGAROLYTIC SYSTEM**

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**Background**

In a marine carbon cycle, the metabolic pathway of agar is not fully understood. Agar is a recalcitrant hetero-polysaccharide. Many agarases are known to fragment agar polymer into disaccharide unit, neoagarobiose, that is split into two monomeric sugars (D-galactose and 3,6-L-anhydrogalactose). Metabolic fate of 3,6-L-anhydrogalactose is still mysterious.

**Objectives**

We aim to elucidate full repertoire of agarolytic system in nature not only for understanding the marine carbon cycle but also for construct a synthetic agarolytic system for biomass utilization.

**Methods**

Using experimentally verified functional agarolytic genes as a probe, we searched all available microbial genomes in the public databases and collected all microbial genomes having potential agarolytic activity. In comparative genomics approach, we combined three distinct procedures such as ortholog prediction, gene cluster analysis and transcriptomic analysis (RNAseq) of three genomes, *Saccharophagus degradans*, *Marinimicrobium agarolyticum* and *Vibrio* sp. EJY3, we predicted potential genes related to agar metabolic pathways.

**Conclusions**

The predicted gene set from comparative genomics was divided into several functional categories: i) metabolic enzymes, ii) regulatory proteins (e.g. transcription factor), iii) signaling proteins and transmembrane proteins. We identified phylogenetically conserved genes and designated those genes as the core gene set. By functional examination, we revealed new genes essential for 3,6-anhydrogalactose and designed a synthetic *E. coli* system metabolizing agar as a sole carbon source.

**FEMS-2687**

**Biotechnology and industrial microbiology**

**FUNGAL STRAIN DEVELOPMENT FOR IMPROVED PROTEIN PRODUCTION:  
PROTEASE MUTANT APPROACHES AND GENOME MINING FOR NOVEL  
ENZYME DISCOVERY..**

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**Background**

Optimized protein production in filamentous fungi requires the availability of fungal strains with low levels of secreted protease activity in order to improve secreted protein levels, including shelf life. Already for several decades research has been carried out to obtain these type of mutants, leading to the isolation of mutants with very favorable characteristics, one being a mutation in a transcriptional regulatory gene, *prtT* (e.g. Punt et al;., 2008).

**Objectives**

Based on these results further improved strains have been developed using different selection approaches.

**Methods**

Controlled fermentation experiments with selected mutant strains revealed different improved characteristics, whereas full genome sequencing was carried out to identify the genetic basis of the mutant phenotypes. Identification of relevant protease-regulatory genes has also been carried out using collections of regulatory gene knock-out strains in *N. crassa* and *A. niger*.

**Conclusions**

Several examples of the use of selected mutant strains in our research to discover and produce novel hydrolytic enzymes will be presented.

**FEMS-2690**

**Biotechnology and industrial microbiology**

## **DIRECT ETHANOL PRODUCTION FROM BAGASSE CLOSTRIDIUM CELLULOLYTICUM**

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### **Background**

In recent years the direct process of ethanol production from lignocellulosic materials, has been considered greatly due to its efficiency and money-saving manner of manufacturing. Some *Clostridium* species such as *C.thermocellum* sp. and *C.cellulolyticum* sp. has received many attentions because of their extracellular enzyme production ability.

### **Objectives**

The ultimate goal of present study is evaluating the ethanol production ability of *C.cellulolyticum* from untreated bagasse and delignified bagasse as cellulose source. The ability of *C.cellulolyticum* H10 for direct consumption of cellulose, eliminate the hydrolysis processes and lowered the end cost of ethanol production and also decreased the environmental threats of the existing processes.

### **Methods**

In this study the bacterial strain was cultured in modified CM3 culture medium in anaerobic condition and after 20 days the produced ethanol was determined by gas chromatography, then cellulose was replaced with untreated bagasse and the ethanol production was examined. Untreated bagasse was delignified with three different pretreatment methods which include sodium hydroxide, sodium chlorite and acetic/nitric acid and the delignified outputs of all these delignification methods were used as carbon sources for bacterial growth and ethanol production were determined.

### **Conclusions**

Acetic/nitric acid delignification methods showed the highest delignification value with 86.7% lignin content decrease and the highest ethanol per cellulose yield with values of 0.19 (gr<sub>ethanol</sub>/gr<sub>cellulose</sub>).

**FEMS-2710**

**Biotechnology and industrial microbiology**

**LOW CYTOSOLIC PH AS A SIGNAL FOR CELL SURVIVAL IN H<sub>2</sub>SO<sub>4</sub> STRESSED CELLS**

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**Background**

In the process of bioethanol production yeast biomass is recycled using treatment with dilute sulfuric acid to control the bacterial population. This treatment can lead to loss of cell viability, with consequences on the fermentation yield.

**Objectives**

To understand and ideally enhance yeast cell survival in these conditions, we analyzed the functional cellular responses of *S. cerevisiae* to inorganic acid stress.

**Methods**

Was analyzed growth and intracellular pH (pH<sub>i</sub>) in low pH conditions, using the pH-sensitive GFP derivative ratiometric pHluorin expressed in the cytoplasm of the strains.

**Conclusions**

*S. cerevisiae* mutants in cell wall integrity (CWI) and calcium signaling have reduced growth and viability at a low external pH (pH<sub>ex</sub>) of 2.5. WT yeast showed evident defect in growth only at a pH<sub>ex</sub> of 1.5, showing that a functional cell wall biogenesis is crucial for survival. To our surprise, the CWI mutant showed enhanced viability at pH<sub>ex</sub> of 1.5 compared to pH<sub>ex</sub> 2.5. While at a pH<sub>ex</sub> of 2.5 the pH<sub>i</sub> was unaffected compared to pH<sub>ex</sub> 5.0, at pH<sub>ex</sub> 1.5 the pH<sub>i</sub> of a CWI mutant was reduced. This reduction by itself did not lead to loss of viability. Rather, artificial reduction of pH<sub>i</sub> at pH<sub>ex</sub> 2.5 rescued the CWI and Ca<sup>2+</sup> signaling mutants. Also, the lethal effect of low pH<sub>ex</sub> on CWI and Ca<sup>2+</sup> mutants takes place only in growing cells. However, a lowered pH<sub>i</sub> leads to a reduction in growth rate, and thus protects the cells from death. Likely, cytosolic pH is a signal that directs the growth-stress tolerance trade-off in yeast.

**FEMS-2725**

**Biotechnology and industrial microbiology**

**PRELIMINARY STUDY ON ANTIMICROBIAL ACTIVITY OF BACTERIAL  
SIDEROPHORE**

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**Background**

Bacteria must acquire iron by competing with environmental chelation. One mechanism for bacterial iron acquisition utilizes siderophores. These molecules are small-molecule chelating agents that have been isolated from many microorganisms. As a current study, siderophore is used to deliver an antibiotic payload, through production of siderophore linked antibiotics e.g. salmycins.

**Objectives**

In this study we aimed siderophore production from soil bacteria and detection of their antimicrobial activity .

**Methods**

Bacteria was isolated from soil samples. For detection of siderophore existence in these bacteria, Cas agar medium was used. Identification of siderophore producer bacteria were used 16srRNA analysis. The crude siderophore was produced and extracted from different soil bacteria. Antimicrobial effect of siderophore was determined on several pathogens (*Candida albicans* ATCC 10231, *Pseudomonas aeruginosa* 27853, *E.coli* 25922, *Staphylococcus aureus* 25923, *Bacillus subtilis* 6633, *Proteus mirabilis*, *Enterobacter aerogenes*).

**Conclusions**

As a result of study, we were observed that siderophore from soil bacteria have antimicrobial effect on pathogen microorganisms. Nowadays, These studies are thought as a new methods against the growing antibiotic resistance. Mimicry of Iron-uptake dependent mechanism will can be new approach in medicine.

**FEMS-2727**

**Biotechnology and industrial microbiology**

**PSEUDOMONAS PUTIDA GENOME ANALYSIS: REPEAT SEQUENCES, MEGAPLASMID AND SOLVENT TOLERANCE REVISITED**

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**Background**

Solvent tolerant strains of the ubiquitous soil bacterium *Pseudomonas putida* are increasingly important for a wide range of industrial biotechnology applications. Because of its remarkable solvent and stress tolerance and its energy efficiency, *P.putida* S12 has been described as an important strain for production of value added aromatic compounds and for efficient whole cell biotransformation of HMF to FDCA (1, 2, 3, 4).

**Objectives**

We have undertaken detailed genome sequence analysis to identify and understand the background of solvent tolerance and energy efficiency in *P.putida* S12.

**Methods**

Whole genome sequencing was performed through Illumina HiSeq and PacBio RSII sequencing. Assembly was performed using the CLC Genomic Workbench (BLASR, SSPACE-LongRead scaffolder, Gapfiller version 1.10) and manual closure of remaining gaps. The completed genome sequence was subject to automated annotation by the NCBI Prokaryotic Genome Annotation Pipeline with manual annotation of prominent *Pseudomonas* features.

**Conclusions**

The full *P.putida* S12 genome consists of a 5.8 Mb circular genome and a 557 kb circular megaplasmid. Both the genome and the megaplasmid contain various mobile elements among which multiple copies of the ISS12-type repeat sequence (5). Location of ISS12 repeats and number variation in various strains indicate a role in acquisition and maintenance of solvent tolerance. In addition, gene composition of the megaplasmid indicates an essential role in instigation of solvent tolerance and resistance to other stress conditions. Further comparative analysis of the S12 genome will be presented together with functional analysis of predicted gene and



metabolic functions.

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**FEMS-2776**

**Biotechnology and industrial microbiology**

**EFFECT OF SILENCING ASPERGILLUS TERREUS YAP1 GENE, ON REACTIVE OXYGEN SPECIES (ROS) ACCUMULATION PATTERN AND LOVASTATIN BIOSYNTHESIS.**

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**Background**

In an earlier work we found a ROS build up during lovastatin production phase, which coincided with *sod1* gene (oxidative stress defense enzyme) down regulation. Later, we showed that ROS regulate lovastatin biosynthetic genes. The mechanism by which ROS induce these genes is unknown, but it is considered to be through oxidative-stress-response transcription factor(s). Expression profiles suggested that *A. terreus* Yap1 is a candidate, although probably as a negative regulator. These findings could be used to design new genetic improvement methods.

**Objectives**

Study the role of Yap1 in the control of ROS and in lovastatin biosynthesis, in liquid submerged fermentation (SmF) and in solid-state fermentation (SSF). Also investigate if the perturbation in the ROS profile (caused by silencing *yap1*) can increase lovastatin production.

**Methods**

Silencing vector was constructed by ligation of a fragment of *Atyap1* gene to pGdpPki-RNAi vector. *A. terreus* TUB F-514 transformants were characterized. Lovastatin was quantified by HPLC, and ROS concentration by diclorofluorescein and gene expression by Northern Blot.

**Conclusions**

- 1) *yap1* silencing in *A. terreus* also caused decreased expression of gene *sod1*.
- 2) ROS build up began before schedule, reaching higher levels than in the parental.

Hence,

3) Gene *brlA* (regulator of conidiation) expressed earlier, and reached higher spore densities.

4) Also, gene *lovE* (lovastatin genes specific transcription factor) expression and lovastatin production started earlier and reached higher production levels than the parental in both culture systems. *yap1*-silenced mutants displayed lovastatin production increases of: 60% in SmF and 70% in SSF.

**FEMS-2822**

**Biotechnology and industrial microbiology**

**GENOME SHUFFLING OF CLOSTRIDIUM BEIJERINCKII DSM 6423 FOR  
IMPROVED ISOPROPYL ALCOHOL PRODUCTION**

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**Background**

The limited supply and the negative environmental effects of the use of petroleum-derived fuels and chemicals have stimulated efforts for the development of more environmentally-friendly processes.

**Objectives**

The bacterial fermentation of carbohydrates is a promising way for the production of green chemicals and biofuels. My project aims at improving the efficiency of the anaerobic conversion of sugars by *Clostridium* species into C3 alcohols, namely isopropanol.

**Methods**

Genome sequencing and fermentation performance studies were carried out on multiple *Clostridia* strains including the natural Isopropanol-Butanol-Ethanol producing strain *Clostridium beijerinckii* DSM 6423 (NRRL B593). This strain was then used to generate, through N-methyl-N'-nitro-N-nitrosoguanidine (NTG) mutagenesis, 36 strains with altered fermentation profiles. The selective growth was performed in the presence of 3 suicide substrates or a high concentration of isopropanol (> 35g/L). Further improvement of this first set of mutated strains was achieved through genome shuffling which uses recursive protoplast fusion and offers the advantage of entire genome recombination (Gao, Zhao, Zhang, He, & Jin, 2012). Screening of the final strains was based on enhanced isopropanol tolerance, the best of them being able to tolerate concentrations of up to 50g/L.

**Conclusions**

Some improvement in isopropanol production compared to the wild type strain was attained with strains showing higher solvent yields and/or better selectivity. The best *Clostridium* strains obtained produce less butanol and more isopropanol allowing a more efficient bioprocess.

**FEMS-2827**

**Biotechnology and industrial microbiology**

**EVALUATION OF OENOCOCCUS OENI EXOPOLYSACCHARIDES AS ENDOGENEOUS PROTECTIVE AGENTS FOR MALOLACTIC STARTER PRODUCTION**

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**Background**

The malolactic fermentation (FML) is an essential winemaking step driven by lactic acid bacteria. It takes place after the alcoholic fermentation and mainly consists in the conversion of L-malic to L-lactic acid, with subsequent improvement of the wine microbial stability, aroma and flavor. *Oenococcus oeni* is the most suitable species for driving FML in wine. *O. oeni* may come from the indigenous microflora found on grapes or in the cellar, or it may be massively added to wine as a malolactic starter. *O. oeni* malolactic starters are currently selected for their resistance to wine and their ability to produce polysaccharides is so far not considered. However, this property could be crucial for the protection of the bacteria during starter production and use.

**Objectives**

Our main objective was to examine whether the stimulation of the production of exopolysaccharides by the bacteria *in situ* before freeze drying could protect the bacteria and improve the survival rates towards productions steps but also during inoculation in wine.

**Methods**

The genome sequencing of 50 *O. oeni* strains permitted us to realize the inventory of the exopolysaccharides genes (RAST, Kaas). The exopolysaccharides analysis included the quantification (colorimetric and analytical methods) and phenotypic observation (optical and electron microscopy) of the produced exopolysaccharides. The production of *O. oeni* strains in a lyophilized form and their later inoculation in wine (freeze-drying, cells viability).

**Conclusions**

We observed that the tested strains showed specific survival behaviors, which were perfectly correlated with their EPS biosynthetic ability. The protecting role of the bacterial exopolysaccharides is now to be proposed at industrial level.

**FEMS-2869**

**Biotechnology and industrial microbiology**

## **GLYCEROL PRE-TREATMENT OF SUGAR CANE BAGASSE AND EFFECT ON SUBSEQUENT ENZYME HYDROLYSIS**

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### **Background**

**Background:** The second generation ethanol using agro-industrial waste is a promising alternative biofuel but the challenge is a technological approach to deconstruction the recalcitrant lignocellulose.

### **Objectives**

**Objective:** The present work aimed to study the effect of pre-treatment of sugar cane bagasse with microwave radiation in the presence of glycerol on chemical composition and the efficiency of subsequent enzymatic hydrolysis.

### **Methods**

Bagasse immersed in glycerol 10% were subjected to 2 min of microwave irradiation and the solid fractions resulting were used in the analysis of fibers, TGA, DTG, DSC, FTIR, X-ray and transmission electron microscopy and the liquid fractions were used to determine sugar and phenol contents. Samples of bagasse treated and untreated (control) were submitted to enzymatic hydrolysis for 24 to 72 h at 55 °C with enzymatic solutions obtained by the cultivation of *M. thermophila* M.7.7.

### **Conclusions**

Infrared spectra and thermal analysis showed that pre-treatment acted on the lignin and hemicellulose of bagasse. The hydrolysis with the enzyme produced by *M. thermophila* supplemented with  $\beta$ -glucosidase, afforded 74.0 mg/g and after 72 h the highest yield of reducing sugars was 240.9 mg/g, with  $\beta$ -glucosidase or not. The pre-treatment with microwave and glycerol improve in 40% the enzymatic hydrolyses of the cellulose.



**FEMS-2936**

**Biotechnology and industrial microbiology**

**FUNGAL AND BACTERIAL CELLULOLYTIC ENZYMES TRCEL7B AND CFXYN11A ACTING IN SYNERGY DURING THE HYDROLYSIS OF SUGAR CANE BAGASSE**

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**Background**

Enzymatic bioconversion of lignocellulose to sugar monomers is performed by cellulases and hemicellulases acting synergically. Synergy among cellulases has been documented [1]; synergy between cellulases and accessory enzymes has been studied in cellulosome systems from *Clostridium cellulovorans* [2]. *Trichoderma reesei* and *Cellulomonas flavigena* are two not related cellulolytic organisms which produce enzymes with reported cellulase and xylanase activities, respectively[3,4]

[1] Appl. Biochem. Biotechnol. 2002. 101, 41-60.

[2] Bioresour. Technol. 2010101, 4472-4478.

[3] J. of Biotech 2001.87,179-191

[4] Bioresour.Technol. 2010. 101, 5539–5545.

**Objectives**

To evaluate synergy between two not complex enzymes, fungal cellulase (*TrCel7B*) and bacterial xylanase (*CfXyn11A*), in the sugar release of enzymatic hydrolysis of sugar cane bagasse.

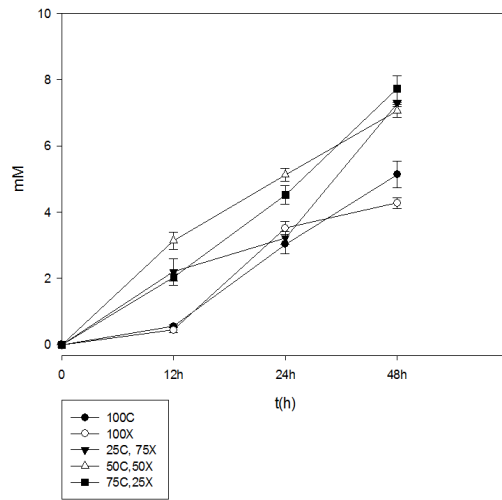
**Methods**

Full length *CfXyn11A* xylanase from *C. flavigena* was expressed in *E.coli* [4]. Core *TrCel7B* was expressed in *T.reesei* [3]. Enzymatic bagasse hydrolysis was performed in equimolar ratios of cellulase(C) and xylanase(X): 100C,75C:25X,50C:50X,25C:75X, 100X. Release of sugar monomers was followed by HPLC after acid hydrolysis. Degree of synergy(D.S.) was quantified as the ratio of the specific activities of the enzymes in the hydrolysis mixtures divided by the sum of the individual specific activities at the concentration used.

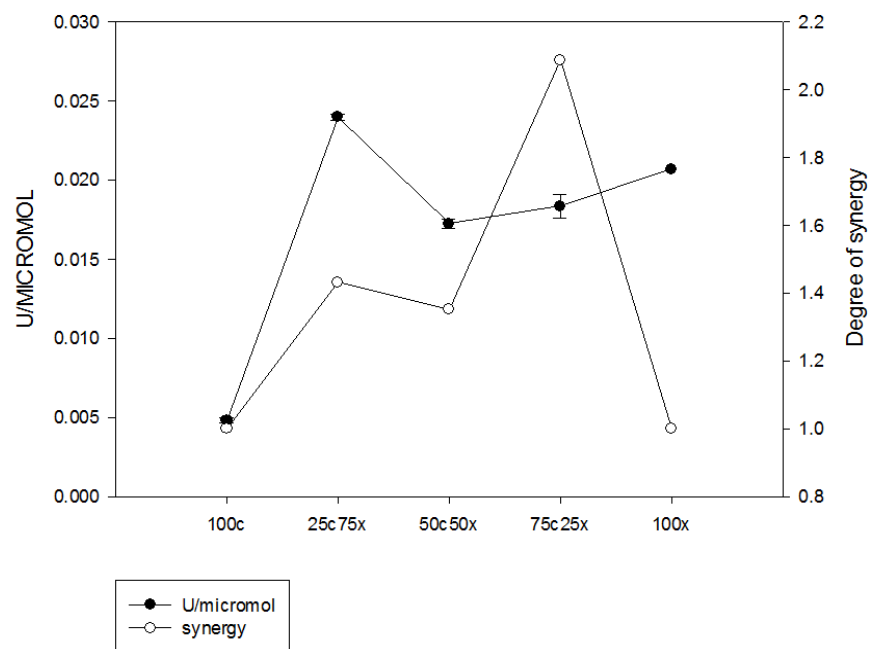
**Conclusions**

Synergy between *CfXyn11A* and *TrCel7b* during bagasse hydrolysis was observed in glucose and xylose but not in arabinose release. The highest xylose and arabinose release observed at 12 h suggests a debranching role of the xylanase prior to cellulase hydrolysis occurring mainly after 24 h. However, a high D.S. observed in the 50C:50X at 12h, enforce the best performance of enzymes when they act together.

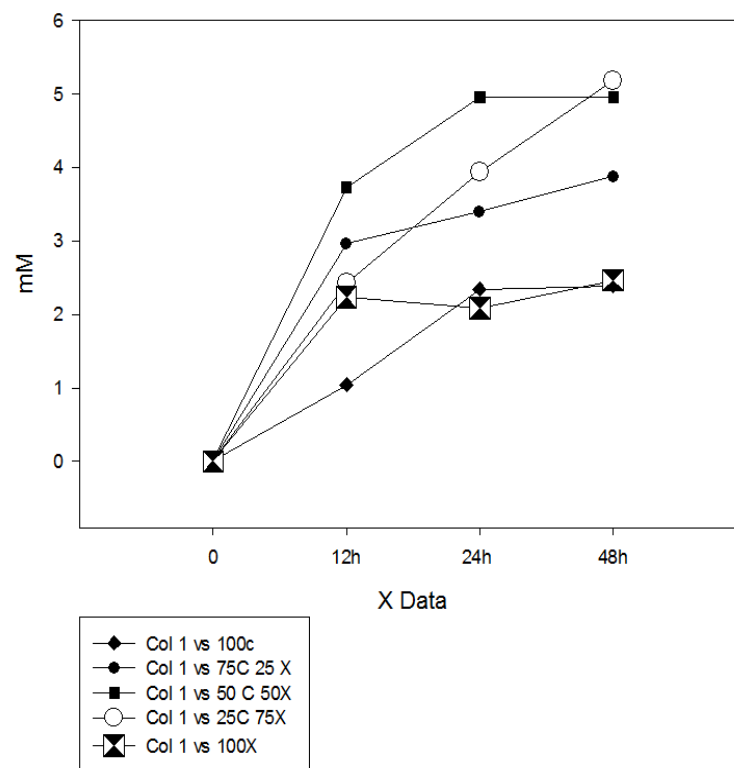
Glucose release



Synergy xylose release 12h



### Xylose release





**FEMS-2950**

**Biotechnology and industrial microbiology**

**DEVELOPMENT OF YARROWIA LIPOLYTICA AS AN OLEAGINOUS CELL  
FACTORY PLATFORM FOR PRODUCING NUTRACEUTICAL AND COSMETIC  
MATERIALS**

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**Background**

*Yarrowia lipolytica* which has been extensively used as a model oleaginous yeast for producing heterologous proteins has capability to accumulate lipids (lipidbody, peroxisome etc) up to 50% of the DCW (dry cell weight). In addition, *Y. lipolytica* can be easily manipulated in gene deletion and insertion because it possesses a haploid genome and its genome sequence was completed and it is also known as GRAS (generally recognized as safe). Therefore, *Y. lipolytica* can be a high potential host strain in producing oleaginous (hydrophobic) and high value-added compounds used in nutraceutical and cosmetic materials which are hydrophobic and produced from plants by conventional extraction processes.

**Objectives**

In this study, we aim to construct basic and potential platform strain suitable for production of nutraceutical and cosmetic materials by using *Y. lipolytica*.

**Methods**

We used *Y. lipolytica* Po1g and constructed an ura3 auxotroph strain, *Y. lipolytica* (PolgDura3), in order to use URA3 blaster system which is a useful tool to carry out repeat genetic deletion and insertion on the genome. In addition, we constructed a *Y. lipolytica* (PolgDku70Dura3) in which Ku70 involved in non-homologous recombination was deleted to increase recombination efficiency in this strain.

**Conclusions**

We confirm that *Y. lipolytica* (PolgDku70Dura3) showed higher homologous recombination efficiency (>5 folds) than *Y. lipolytica* (PolgDura3). Next, we are introducing foreign genes involved in biosynthesis of some specific nutraceutical and cosmetic materials into *Y. lipolytica* (PolgDku70Dura3). This strain and genetic tool will be very useful to construct various customized strains for producing nutraceutical and cosmetic materials.

**FEMS-2954**

**Biotechnology and industrial microbiology**

**MODULATION OF OXIDATIVE FOLDING TO IMPROVE RECOMBINANT PROTEIN PRODUCTION IN THE YEAST *SACCHAROMYCES CEREVISIAE***

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**Background**

The yeast *Saccharomyces cerevisiae* is widely used in biotechnology for the production of both bulk chemicals, like ethanol and organic acids, and higher-value compounds such as recombinant proteins, like pro-insulin. Recombinant protein production in *S. cerevisiae* induces different stress responses, including oxidative stress response. This is especially the case when the overall protein folding rate and the oxidative folding rate, i.e. the formation of di-sulfide bonds, are not properly balanced (1).

**Objectives**

In the present study we aim to develop metabolic engineering strategies to increase recombinant protein production by specifically adjusting oxidative folding.

**Methods**

Two industrially relevant proteins were selected based on different overall folding rates: pro-insulin and alpha-amylase. The oxidative folding of these proteins was modulated by A) altering the expression levels of several key players in oxidative folding, e.g. the thiol oxidases Ero1 and Erv2, and B) changing the number of possible di-sulfide bonds, i.e. the number of cysteine residues present.

**Conclusions**

The effects of these modulations on reactive oxygen species (ROS) levels, unfolded protein response, oxidative state, protein titers and overall physiology are monitored under strictly controlled growth conditions.

**Reference**

1. Tyo K.E., Z. Liu, D. Petranovic, J. Nielsen (2012) Imbalance of heterologous protein folding and disulfide bond formation rates yields runaway oxidative stress. BMC Biol. 10:16



**FEMS-2969**

**Biotechnology and industrial microbiology**

## **PRODUCTION OF BIO-OIL BY OLEAGINOUS YEAST**

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### **Background**

The Dutch government is stimulating the transition to a more biobased economy. Microbial oil can be produced from biomass in a green and sustainable way without competing to food crops by using oleaginous microorganisms such as yeast, fungi, bacteria and microalgae. At the HAN BioCentre a unique robust oil producing yeast strain has been selected called HBC025. The strain grows on a broad range of C5 and C6 sugars. The oil is accumulated by HBC025 in the form of triacylglycerols (TAGs) containing predominantly oleic (18:1), linoleic (18:2) and palmitic (16:0) acid. The produced microbial oil can be used as biodiesel but also in other applications such as paint, roads, floors and cosmetics.

### **Objectives**

Objective is the development of an economically feasible fermentation and extraction process for second generation oil production by HBC025.

### **Methods**

A fed-batch fermentation process was developed on glucose as model substrate. Hydrolyzed paper pulp was obtained from Parengo (Renkum, The Netherlands). Extraction methods tested were homogenization and enzyme assisted auto-lysis.

### **Conclusions**

Maximum productivity on glucose as substrate was 0.5 g bio-oil/l/h, which is comparable to the best lipid producing strains in literature (Ageitos et al. 2011). HBC025 was also able to grow on hydrolyzed paper pulp as substrate with the same growth rate. Calculations showed that the process can be economically feasible only in case components from the yeast biomass are also marketed besides the yeast oil.

### **References**

Ageitos JM et al. 2011. Oily yeasts as oleaginous cell factories. *Appl Microbiol Biotechnol* **90**: 1219-1227.

**FEMS-2973**

**Biotechnology and industrial microbiology**

**REVERSIBLE PSEUDOHYPHAE FORMATION IN PICHIA PASTORIS DURING HETEROLOGOUS PROTEIN PRODUCTION.**

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**Background**

The methylotrophic *Pichia pastoris* yeast expression system is widely used for production of heterologous proteins. However, till now morphological changes have not been reported in *P.pastoris*. This study reports for the first time, a comprehensive characterization of Granulocyte colony stimulating factor (GCSF) expression in filamentous *P. pastoris*.

**Objectives**

To investigate the morphological changes in *P. pastoris* during protein production phase.

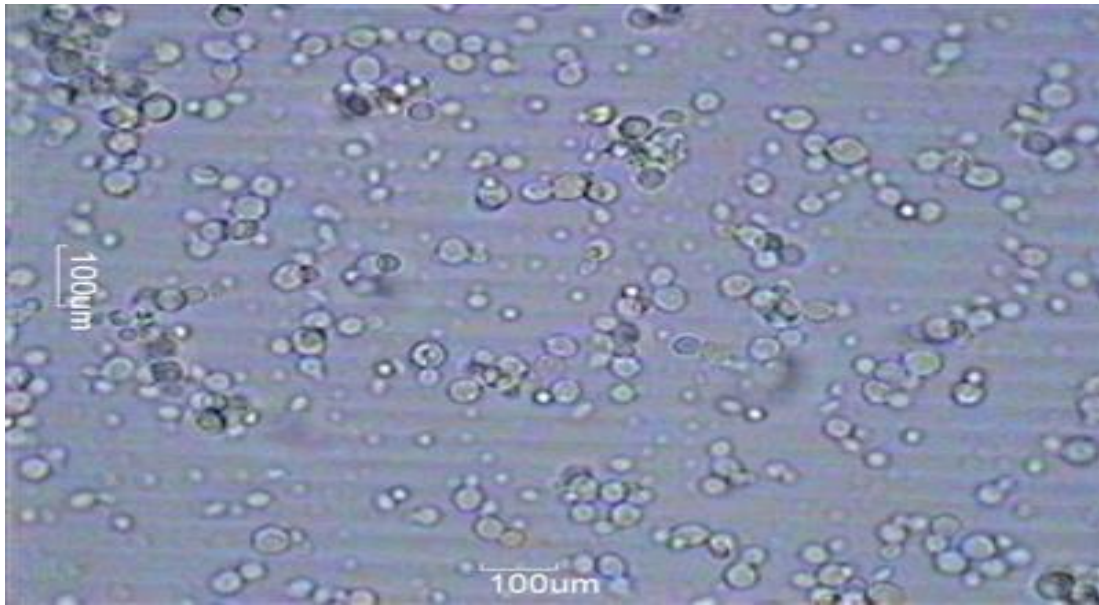
**Methods**

Mut<sup>s</sup> transformants, containing the GCSF-cDNA fused with modified kex2 cleavage site (in the  $\alpha$ -factor secretion signal) under the control of the *alcohol oxidase 1* promoter, were used in the study. The transformants were cultivated in BMGY (buffered glycerol complex medium) followed by BMMY (buffered methanol complex medium). Extracellular protein was quantitated by gel densitometry from 20-fold concentrated culture supernatant. A combination of methanol level and feeding strategy was developed to increase productivity of GCSF at fermenter level.

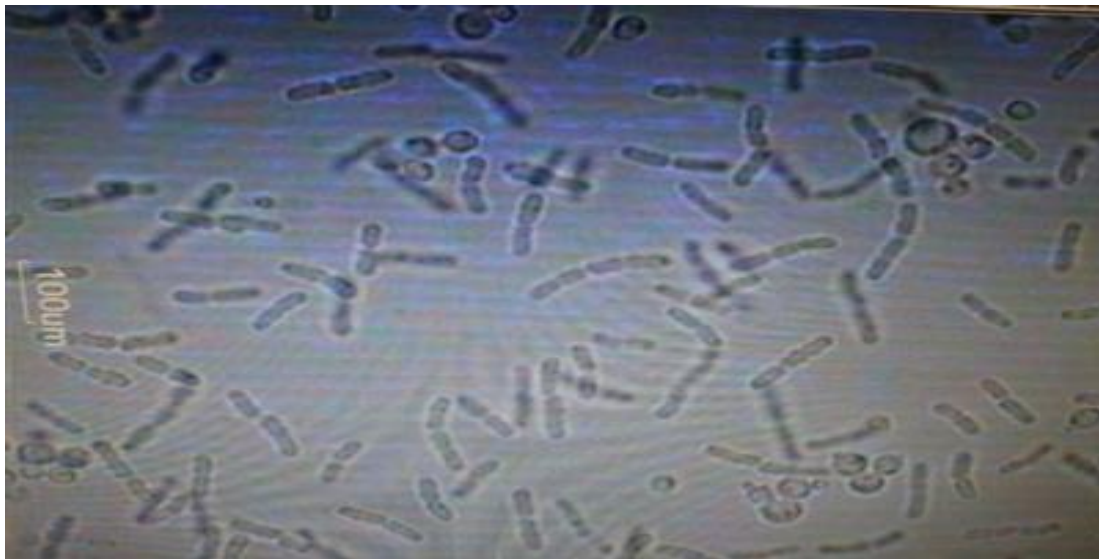
**Observation:**

Reversible Pseudohyphae was observed, resulting in increased surface to volume ratio with an increase in extracellular protein relative to the normal phenotype of spherical cells at same cell dry weight.

A.



B.



**Fig1.: Morphology of recombinants (A) Mut<sup>+</sup>(methanol utilization plus) and (B) Mut<sup>s</sup> (methanol utilization slow) strains of *P. pastoris*, SMD1168 cultivated in BMMY media + 0.4% histidine.**

## Conclusions

*P. pastoris* initiates a striking developmental transition to filamentous form, more so in the Mut<sup>s</sup> phenotype, that results in more surface to volume ratio, and affects productivity of the culture. To the best of our knowledge, data presented here opens the possibility of a filamentous form of *P. pastoris* for production of heterologous proteins.



**FEMS-2982**

**Biotechnology and industrial microbiology**

## **HIGH-LEVEL PRODUCTION OF MONO-COMPONENT AND ENZYME MIXTURES IN MYCELIOPHTHORA THERMOPHILA**

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### **Background**

The fungus *Myceliophthora thermophila* C1 was developed into an efficient and versatile platform for high-level production of industrially relevant enzymes.

### **Objectives**

Through strain development strategies, two strain lineages were obtained that are currently being developed and exploited as production hosts. One lineage (HC) is able to produce and secrete high amounts of enzyme mixtures that contain large amounts of (hemi-) cellulases. The other lineage (LC) is impaired in its cellulase producing capability, resulting in low background-protein production. The LC strain has been further developed for high-level enzyme production. Total protein levels up to 80 g/L has been reached of which ~ 80% correlates to the introduced enzyme.

### **Methods**

By transforming the LC strain with selected C1 genes, a wide collection of strains was obtained, each of which produced mainly one enzyme. This has led to an enzyme library of over 100 functional enzymes of which many have been purified and characterized. The LC strain can also be designed and constructed in such a way that it produces only those enzymes that are functional under dedicated application conditions.

### **Conclusions**

In conclusion, *M. thermophila* was developed into a high-level protein-production platform. The HC strain is successfully applied to produce enzymes for the production of biofuels and biobased-chemicals. The LC strain is being used to produce single enzymes and defined combinations of enzymes. The obtained C1-enzyme library is a rich source for academic and industrial research. The properties of *M. thermophila* C1

make this fungus a highly suitable alternative for traditional fungal protein production hosts.

**FEMS-2999**

**Biotechnology and industrial microbiology**

**NEW  $\phi$ BT1 SITE-SPECIFIC INTEGRATIVE VECTORS WITH NEUTRAL PHENOTYPE IN STREPTOMYCES**

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**Background**

Integrative plasmids are one of the best options to introduce genes in low-copy-number and a stable form into *Streptomyces*, but they generate mutations in their integration sites. Phage (PHI)C31, the most common integrative site used in *Streptomyces*, integrates at different positions (*attB* and pseudo-*attB* sites) in the *Streptomyces* genome generating different mutations and phenotypes. The less common integration site (PHI)BT1, integrates at a unique *attB* site localized in gene SCO4848 (*S. coelicolor* genome) or their homologues in other streptomycetes.

**Objectives**

Constructing an integrative plasmid with neutral phenotype.

**Methods**

Four plasmids were created modifying the  $\phi$ BT1 integrative vector pMS82: pNG1, in which the SCO4849 was introduced under the control of the promoter region of SCO4848 into pMS82 to restore the phenotype generated by the integration of the plasmid; pNG2, in which the promoter P<sub>ermE</sub><sup>\*</sup> was introduced into pNG1 to facilitate heterologous gene expression; pNG3 in which the *bla* gene for ampicillin resistance was included into pNG1 in order to facilitate selection in *E. coli*; and pNG4, in which the *bla* gene was introduced into pNG2.

**Conclusions**

The plasmids pNG1, pNG2, pNG3, and pNG4, are the only integrative vectors designed to produce a neutral phenotype when they are integrated in streptomycetes.



**FEMS-3020**

**Biotechnology and industrial microbiology**

**THE INFLUENCE OF LACTIC ACID BACTERIA ADDITIVE ON THE LEVEL OF RESIDUAL WATER-SOLUBLE CARBOHYDRATES IN CORN SILAGE**

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**Background**

Ensilaging is a high moisture crop conservation method based on the fermentation of water soluble carbohydrates (WSC) guided by epiphytic microflora, specifically by lactic acid bacteria (LAB). LAB additives (inoculants) in ensilaging are used for promotion of optimal fermentation and rapid pH drop thus improving dominant nutrients preservation. However, the impact of inoculation on the level of residual WSC, which in high levels may result in silages aerobic deterioration, is not clear.

**Objectives**

During the present study, the application of LAB inoculant on residual WSC was investigated.

**Methods**

The three different yellow corn hybrid (Bc 418b, Bc 678 and Bc exp 6) produced in the same production conditions (31,01 – 38,52% DM) were ensiled in five replications in laboratory scale silos with and without Sil-All®4x4 LAB inoculant in a concentration  $1 \times 10^7$  CFU/g of fresh material. The silages were sampled on the 21<sup>st</sup> and the 60<sup>th</sup> day. The contents of lactic acid, WSC and pH were monitored.

**Conclusions**

The analyses showed that the inoculated silages had a significant increase ( $P < 0,05$ ) of lactic acid concentrations (g/kg DM basic) both on the 21<sup>st</sup> (31,45) and the 60<sup>th</sup> day (33,28) in contrast to silages without inoculant (21<sup>st</sup> 22,49; 60<sup>th</sup> 26,38). The same pattern was observed with pH. At the end of ensiling the difference in WSC content between silage with (8,63 g/kg DM) and without inoculant (7,39 g/kg DM) was not statistically significant ( $P > 0,05$ ). Inoculation of silages with Sil-All®4x4 stimulates lactic acid production and rapid pH drop whereas has no influence on the level of residual water-soluble carbohydrates.

**FEMS-3059**

**Biotechnology and industrial microbiology**

## **FUNCTIONAL ANALYSIS OF FILIPIN TAILORING GENES FROM STREPTOMYCES FILIPINENSIS**

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<sup>2</sup>Organic Chemistry, Universidad Autónoma de Madrid, Madrid, Spain

### **Background**

*Streptomyces filipinensis* is the industrial producer of filipin, a 28-membered non-glycosylated pentaene macrolide widely used for the detection and the quantitation of cholesterol in biological membranes.

### **Objectives**

Identification of tailoring genes belonging to the biosynthetic cluster for the biosynthesis of filipin, and generation of engineered derivatives for pathway elucidation.

### **Methods**

A region of 13778 base pairs of DNA from the *S. filipinensis* genome was isolated, sequenced, and characterized by gene replacement and complementation techniques.

### **Conclusions**

This set of genes shows synteny with the homologous *pte* genes from the filipin cluster of *S. avermitilis*, and includes two cytochrome P450 monooxygenase encoding genes, *filC* and *filD*, which are proposed to catalyze specific hydroxylations of the macrolide ring at C26 and C1' respectively. Gene deletion and complementation experiments provided evidence for their role during filipin III biosynthesis. Filipin III derivatives were accumulated by the recombinant mutants at high yield. These have been characterized by mass spectrometry following high-performance liquid chromatography purification thus revealing the post-polyketide steps during polyene biosynthesis. Two alternative routes lead to the formation of filipin III from the initial product of polyketide synthase chain assembly and cyclization filipin I, one through filipin II, and the other one through 1'-hydroxyfilipin I, both compounds being biologically active.



**FEMS-3065**

**Biotechnology and industrial microbiology**

## **SCREENING OF LACTIC ACID BACTERIA FOR PRODUCTION OF BIOSURFACTANTS**

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### **Background**

Lactic acid bacteria (LAB) have been extensively used for thousands of years in food fermentation and nowadays in the industrial production of lactic acid and other metabolites.

### **Objectives**

In this study, the biosynthetic potential of lactic acid bacteria was exploited for biosurfactant (BS) production.

### **Methods**

66 Lactobacilli strains were screened for BS production when growing in different MRS-based media at static conditions. To determine BS production (extracellular and cell-bound), surface tension (ST) of supernatant and phosphate buffered saline (PBS) extract of the strains were measured by tensiometer. To determine critical micellar concentrations (CMC), PBS extracts of the strains remarkably reduced ST were diluted.

Pyrene solubilization assays were further done as a complementary assay to prove the presence of BSs. 1 mg of pyrene was distributed into glass test tubes and was subjected to supernatant and PBS extract of the strains in shaking conditions for 24h. The values were then compared with those of pyrene solubilization at the presence of rhamnolipid in different CMCs.

### **Conclusions**

The results showed there is a significant potential for BS production among the strains screened and 9 out of 66 Lactobacilli strains reduced ST of PBS extract between 19 and 22 units. The values are close to the data reported by Moldes *et al.* 2007 for *Lactobacillus pentosus* CECET-4023 (20 units) and Gudina *et al.* 2011 for *Lactobacillus paracasei* spp. *paracasei* A20 (22 units).

Pyrene solubilization assay can be taken into account as a promising method to identify BS-producing strains.

Concentrations and characterization of BSs produced are under investigation.

**FEMS-3066**

**Biotechnology and industrial microbiology**

**CHARACTERIZATION OF SCO4439, A D-ALANYL-D-ALANINE CARBOXYPEPTIDASE INVOLVED IN SPORE CELL WALL MATURATION, SPORE RESISTANCE, GERMINATION AND MYCELIAL RESISTANCE TO VANCOMYCIN AND TEICoplanin**

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**Background**

Streptomycetes are model microbes with complex developmental cycles. The last advances in system biology methodologies allowed the identification of large datasheets of proteins and genes differentially expressed during development whose biological function remains, in many cases, unexplored

**Objectives**

Characterization of the biological function of SCO4439, a developmentally associated D-alanyl-D-alanine carboxypeptidase (DD-CPase) overexpressed during the aerial mycelium and sporulation stages.

**Methods**

Strain: *Streptomyces coelicolor* M145

SCO4439::Tn5062 mutant was made according to **Fernández-Martínez (2011)**.

**Antonie Van Leeuwenhoek 99:515-522.**

**Conclusions**

1- SCO4439 is a multidomain protein harbouring a DD-CPase at its carboxyl end, and a putative cytosolic transcriptional regulator domain at the N-terminal end. Both domains are separated by a putative transmembrane region.

2- The DD-CPase activity was demonstrated '*in vitro*' to have a preference for DAla-DAla peptides, and to be inhibited by penicillin G.

3- SCO4439 was demonstrated '*in vivo*' to be involved in the resistance of the spores to heating and acid, in the spore swelling during germination, and in the resistance of the mycelium to glycopeptide antibiotics (vancomycin and teicoplanin).

4- The DD-CPase domain, together with the putative hydrophobic transmembrane region are highly conserved in *Streptomyces*, and the presence of both domains is essential to restore the phenotypes observed in the SCO4439::Tn5062 mutant. The conservation of the putative transcriptional regulator domain is much lower, and it is not necessary to restore the phenotypes detected in the SCO4439::Tn5062 mutant.

5- **SCO4439 controls PG cross-linking** releasing DAla from peptidoglycan lateral chains and reducing the substrate for the transpeptidation.

**6- The level of peptidoglycan cross-linking is regulating spore resistance and germination.**

**FEMS-3090**

**Biotechnology and industrial microbiology**

## **AN EFFICIENT FED-BATCH FERMENTATION PROCESS: THE PRODUCTION OF STREPTOKINASE FROM E. COLI**

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<sup>3</sup>BERPDC, Institute of Microbial Technology, Chandigarh, India

### **Background**

Streptokinase is a therapeutic protein drug of bacterial origin. Its role in thrombolytic therapy has been well established. It is either produced from *Streptococcus*, its natural producer or from *E.coli* by recombinant means. The production from the former, however, is problematic due to low yield and simultaneous production of toxic endolytic chemicals. At IMTECH, the gene encoding for streptokinase from *Streptococcus equisimilis* was cloned and expressed in *E. coli* BL21 (DE3). Initially the expression in the latter was very low, hence the coding sequence was appropriately modified using molecular biology approaches and as a result of such optimizations, a high expression level of upto 80 mg l<sup>-1</sup> was obtained in shake flask cultivation.

### **Objectives**

The main objective of this study was to develop a bioprocess that could be used for mass scale production and purification of this high value therapeutic protein.

### **Methods**

To achieve the above objective, a fed-batch fermentation process was developed and optimized. Cultivation parameters like dissolved oxygen concentration (DOC), pH, media constituents and feed rate were carefully optimized and product yield was monitored.

### **Conclusions**

The process optimization resulted in an intracellular expression level of 1120 mg l<sup>-1</sup> in high cell density fermentation. This represented a 14-fold increase in production levels from flask to bio-reactor. Also the specific expression level *i.e.* gram protein produced per gram wet cell mass remained unchanged. This yield is the highest reported so far for recombinant Streptokinase.



**FEMS-3100**

**Biotechnology and industrial microbiology**

**UNRAVELING BROWN ROT FUNGAL LIGNOCELLULOSE DEGRADATION MECHANISMS FOR BIOPROCESSING APPLICATIONS**

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**Background**

This research investigates the mechanism of lignocellulose degradation by brown rot fungi and potential industrial applications of this system. The brown rot non-enzymatic degradative mechanism is largely unexplored for use in biomimetic industrial/biorefinery applications and understanding the degradative mechanisms of brown rot fungi will lead to better methods of addressing the recalcitrance problems associated with lignin and crystalline cellulose in the pretreatment of biomass in biorefinery applications. A chelator mediated Fenton (CMF) system has evolved to substitute for components of the cellulolytic enzyme machinery in the brown rot fungi, generating an alternative efficient mechanism for depolymerization of biomass. The CMF system is unique among biological systems in being the only reported substrate deconstruction system based on oxygen radical chemistry that permits non-enzymatic deconstruction at a considerable distance (several microns) from the organism.

**Objectives**

Specific objectives of this research include: 1) Examining the morphology of the fungal sheath in a brown rotted fungal-wood complex to determine how the sheath may aid in the movement of CMF components and enzymes; and 2) Analyzing the structure of lignocellulose in early and late-stages of brown rot fungal degradation to assess how the CMF system modifies the wood cell wall non-enzymatically.

**Methods**

This research utilized several different techniques including the ORNL Bio-SANS (small angle neutron scattering) beam line to obtain the mechanistic information necessary to better characterize processes involved in non-enzymatic biodegradation of lignocellulose by brown rot fungi.

**Conclusions**

This research supports a CMF model system being employed by brown rot fungal organisms in lignocellulose deconstruction.

**FEMS-3106**

**Biotechnology and industrial microbiology**

## **CHARACTERIZATION OF 3-KETOSTEROID-9 $\alpha$ -HYDROXYLASES IN RHODOCOCCUS RUBER STRAIN CHOL-4**

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### **Background**

Bacterial catabolism of steroid compounds provides a broad range of intermediaries needed for the synthesis of pharmaceutical steroid drugs such as 4-androsten-3,17-dione (AD) and 1,4-androstadiene-3, 17-dione (ADD). *Rhodococcus ruber* strain Chol-4, isolated from a sewage sludge sample, is able to grow in minimal medium supplemented with steroids, showing a large catabolic capacity.

The 3-Ketosteroid 9 $\alpha$ -Hydroxylase (KSH) is a key enzyme in the general scheme of bacterial steroid catabolism; it initiates the opening of the steroid ring by the 9 $\alpha$ -hydroxylation of the C9 carbon of 4-ene-3-oxosteroids (e.g. AD) or 1,4-diene-3-oxosteroids (e.g. ADD), transforming them into 9 $\alpha$ -hydroxy-4-androsten-3,17-dione (e.g. 9OHAD) or 9 $\alpha$ -hydroxy-1,4-androstadiene-3,17-dione (e.g. 9OHADD), respectively.

### **Objectives**

Study and characterization of 3-ketosteroid-9 $\alpha$ -hydroxylases in *Rhodococcus ruber* strain chol-4

### **Methods**

The ORF finder and the pDRAW32 programs were used to detect the ORFs. Determination of KSHs induced or constitutive transcription, along with the co-transcription of each cluster, was made by RT-PCR. Mutagenesis of both single, double and triple *kshA* and *kshB* were made by unmarked gene deletion. Growth studies were analyzed on different substrates.

### **Conclusions**

This work presents a set of data that clarifies KHAs specific roles in strain chol-4. We have characterized three different *kshA* and one *kshB* homologous ORFs in strain Chol-4. All of them present constitutive transcription and co-transcription. The mutants have shown differences in growing depending on the substrate.



**FEMS-3142**

**Biotechnology and industrial microbiology**

**TRANSPOSON Tn5-INDUCED MUTAGENESIS OF KANAMYCIN RESISTANCE  
RHIZOBIUM JAPONICUM**

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**Background**

Transposase is an enzyme that binds to the ends of a transposon and catalyzes the movement of the transposon to another part of the genome by a cut and paste mechanism or a replicative transposition mechanism. *Rhizobium* is a genus of Gram-negative soil bacteria, many of which fix nitrogen. Nitrogen fixation is an important part of the nitrogen cycle. Plants cannot use atmospheric nitrogen (N<sub>2</sub>) they must use nitrogen compounds such as nitrates.

**Objectives**

The objective of this research was to determine whether at random transposon mutagenesis could be applied in genetic studies of *Rhizobium japonicum*. One major reason for this is that a range of defined mutations is not available.

**Methods**

In present study *R. japonicum* at frequencies sufficient to allow the isolation of large numbers of insertion mutants. The selection of Tn5 mutants was facilitated by the expression, in all the *R. japonicum* strains we have tested, of the Tn5 encoded kanamycin resistance. A number of auxotrophic and symbiotically defective, single, random transposon (Tn5) mutants were obtained in three slow-growing strains of different DNA homology and serogroups and the single fast growing strain of *R. japonicum*.

**Conclusions**

In conclusion, the diversity of auxotrophs detected and the isolation of symbiotic mutants suggests that transposon Tn5 can be used as a generalized mutagen to isolate a variety of mutants with defects in symbiotic nitrogen fixation. The analysis of such mutants should prove to be useful in elucidating the biochemical, genetic, and regulatory events involved in the *R. japonicum* which effectively nodulates certain Indian soybean cultivars



**FEMS-0988**

**Cell-cell communication / Quorum sensing**

**ACINETOBACTER STRAIN XB174 DEGRADES QUORUM SENSING SIGNAL OF RALSTONIA SOLANACEARUM, PREVENTS WILT AND PROMOTES GROWTH IN EGGPLANT**

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**Background**

*Ralstonia solanacearum* is one of the most destructive plant pathogens and causes bacterial wilt in about 450 plant species worldwide. During infection, the pathogen primarily colonizes plant vascular system in high density leading to wilt and eventually plant death. Expression of virulence factors by the pathogen is regulated by a quorum sensing system activated 3-hydroxy palmitic acid methyl ester (3OH-PAME).

**Objectives**

The objective of this study was to determine 3OH-PAME degradation by bacterial strains isolated from eggplant xylem and to determine their wilt prevention and growth promotion ability in eggplant.

**Methods**

Bacterial strains (n = 6) were earlier isolated from xylem sap of eggplant. Using a bioassay involving indicator *R. solanacearum* strain AW1-3 and High Performance Liquid Chromatography Mass Spectrometry, 3OH-PAME degradation by bacterial isolates was estimated. Strain XB174 degraded 3OH-PAME and was evaluated for wilt prevention and growth promotion in eggplant under greenhouse conditions. XB174 was identified by 16s rRNA gene sequencing and its activity against Tween-20, Tween-80 and tributyrin was determined.

**Conclusions**

Among the strains screened using bioassay, only XB174 degraded 3OH-PAME to 3OH-palmitic acid. Specific activity of crude extracellular enzyme of XB174 towards 3OH-PAME was 17.78 U mg<sup>-1</sup>. XB174 prevented wilt (55.0 ± 7% over control) and improved growth (19.2 ± 10% more growth promotion efficacy over control) in eggplant. XB174 is identified as *Acinetobacter* sp., degraded Tween-20, Tween-80 and tributyrin.

The study reveals the quorum quenching activity of *Acinetobacter* strain XB174 against *R. solanacearum*. After additional screening, extracellular enzyme from XB174 may have applications in agriculture and industries.



**FEMS-2421**

**Cell-cell communication / Quorum sensing**

**FUNCTIONAL CHARACTERIZATION OF A LUXR TYPE REGULATOR, ABAR IN ACINETOBACTER BAUMANNII: INVOLVEMENT OF ABAR IN PATHOGENESIS**

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**Background**

Quorum sensing is a cell to cell communication system that coordinates gene expression in many bacterial species. It has become an increasingly interesting target for the development of alternative drugs for treating multi-drug resistant *Acinetobacter baumannii* infection. However, the role of quorum sensing system in *A. baumannii* has not been characterized.

**Objectives**

The aim of this study is to characterize the functions of AbaR, a putative LuxR type receptor, using an isogenic mutant, and to shed some light on to the development of effective alternative drugs targeting AbaR.

**Methods**

The disruption of *abaR* in *A. baumannii* lead to significant decrease in biofilm formation. In addition, the pellicle production and swarming motility of the *abaR* mutant was substantially decreased compared to that of wild-type. The *abaR* mutant complemented with the intact *abaR* gene reverted back to that of wild-type capable of producing biofilm and displaying swarming motility. The survival rate of mice infected with the *abaR* mutant strain was significantly high than that of those infected with the *A. baumannii* wild-type. Also, whole transcriptome analysis was performed in order to compare the gene expression in wild-type and *abaR* mutant strains. Furthermore, chemical screening was carried out for the possible quorum quenching compounds targeting AbaR.

**Conclusions**

The data from the current study reveals that AbaR plays an important role in *A. baumannii* pathogenesis and this regulator protein could be a putative target of quorum quenching compounds. Supported by KHIDI (grant number: HI14C0257)



**FEMS-2955**

**Cell-cell communication / Quorum sensing**

## **HOW DOES HAMAMELITANNIN INCREASE STAPHYLOCOCCUS AUREUS BIOFILM SUSCEPTIBILITY TOWARDS GLYCOPEPTIDES?**

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### **Background**

*Staphylococcus aureus* biofilm-associated infections are difficult to treat and novel targets are needed to combat these infections. The QS modulator hamamelitannin increases susceptibility of *S. aureus* biofilms towards vancomycin *in vitro* as well as *in vivo*. However, it is unclear how HAM affects susceptibility.

### **Objectives**

Elucidate the mechanism of action of HAM at the molecular level.

### **Methods**

Two parallel strategies were followed. First, we evaluated the effect of HAM on biofilms of *S. aureus* strains with mutations in the QS systems or in genes involved in biofilm formation and virulence. Secondly, using illumina sequencing we identified genes that were differentially expressed in untreated biofilms and biofilms treated with VAN alone or in combination with HAM. Results obtained with both strategies were further investigated using the appropriate tools.

### **Conclusions**

No loss in HAM activity was observed for most of the mutants. In contrast, HAM did not affect biofilm susceptibility of *S. aureus* strains with mutations in the QS system. Using sequencing, we identified a large number of genes that were differentially regulated after treatment (e.g. genes involved in biosynthesis of lysine, D-alanine, glutamine consuming pathways and virulence). This indicates that HAM reduces the upregulation of peptidoglycan biosynthesis normally observed after treatment with VAN. This possibly leads to the increased susceptibility of *S. aureus* biofilm cells towards VAN. Our results further indicate that combination therapy could positively affect morbidity since the upregulation of virulence factors observed for VAN treatment are not observed when VAN is combined with HAM.

**FEMS-0949**

**Cell-cell communication / Quorum sensing**

**ACYL HOMOSERINE LACTONE-BASED QUORUM SENSING AND BIOFILM FORMATION IN THE PHOTOSYNTHETIC BACTERIUM RHODOSPIRILLUM RUBRUM S1H**

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**Background**

MELiSSA (Micro-Ecological Life Support System Alternative) has been conceived as a 5 compartments microorganisms and higher plants recycling system for long haul space flights. *Rhodospirillum rubrum* S1H colonizes compartment II and grows under light anaerobic conditions (LAN) using acetate as carbon source (MELiSSA conditions). Previous work reported that continuous culture of the bacterium in a photobioreactor lead to thick biofilm formation, leading to bioreactor arrest.

**Objectives**

The aim of this research is to investigate the relation of the quorum sensing (QS) system and biofilm formation of *R. rubrum* S1H (wild type, WT) under MELiSSA relevant culture conditions.

**Methods**

In this context we have constructed a mutant strain named M68 that does not produce acyl homoserine lactones (AHLs) signaling molecules. The transcriptomic and proteomic profiles and phenotype of WT and M68 under MELiSSA conditions were compared. In addition, the biofilm development of WT and M68 in a flow cell system under light microaerobic conditions was studied.

**Conclusions**

*R. rubrum* has a cell-to-cell communication system based on AHLs which regulates 8% (326 genes) of the genome of M68. In *R. rubrum* QS regulates pigmentation, photosynthesis, energy generation, carbon metabolism, motility and biofilm formation. To our knowledge this is the first report where QS is linked to biofilm formation in *R. rubrum* S1H under light microaerobic conditions. Further flow cell experiments under MELiSSA conditions will help us to study biofilm formation and to test substances with known anti-biofouling properties.

**FEMS-2148**

**Cell-cell communication / Quorum sensing**

**DETERMINING OF RELATIONSHIP BETWEEN SIDEROPHORE PRODUCTION AND QUORUM SENSING MOLECULES ON MULTIDRUG-RESISTANT ACINETOBACTER BAUMANNII**

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**Background**

In addition to causing various infections, *Acinetobacter* species are described as nosocomial pathogens. These species are increasingly developing resistance against antibacterial agents due to their capability to adapt to any environment. *Acinetobacter* species develops these capabilities against their environment by quorum sensing (QS) mechanism which is a bacterial communication or ambient sensing mechanism. Quorum sensing systems needed for iron uptake comprising genes required for siderophore biosynthesis.

**Objectives**

In this work, effect of signal molecules on siderophore biosynthesis and its relation with QSS in *MDR A.baumannii* were studied.

**Methods**

Twenty strains of MDR *A.baumannii* from different clinical specimens were identified by standard methods. QSS molecules were identified by cross-validation test and siderophore biosynthesis was evaluated on CASagar and thin layer chromatography(TLC). AHL profiles were determined by using *Agrobacterium tumefaciens* biosensor strains (A136 or NTL1). QSS molecules combined with the biosensor strains were separated by TLC from each other.

**Conclusions**

According to results of the study, it was found out that all of the MDR *A. baumannii* strains produced the following miscellaneous QSS molecules in varying amounts: C8-HSL, C10-HSL and C12-HSL and these strains produced hydroxamate- and catechol-type siderophores. Iron uptake in the environments is thought to be a direct relationship with quorum sensing system. The variations in the amounts and types of signal molecules were observed to affect virulence properties. QSS molecules can be used as target molecules in medical therapy. The study provides new information about the virulence factors for the better understanding of the infection properties

of *A.baumannii* in the host.

**FEMS-0594**

**Cell-cell communication / Quorum sensing**

**BIO-CONTROL APPLICATION OF BACILLUS QUORUM SENSING ON AFLATOXIGENIC FUNGI THROUGH THEIR INTER-KINGDOM CROSS-TALK**

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**Background**

Quorum sensing molecules (QSM) are involved in the regulation of complicated processes helping bacterial population benefit from their cell-density. Prokaryotes and eukaryotes' co-evolution raises the prospect of the existence of inter-kingdom signalling pathways. The role of hormone-like molecules in cell communication approves QS role in this cross-talk. Bacterial antagonistic activity against fungi is considered as an inter-kingdom talk. *Bacillus* and *Pseudomonas* have the ability to inhibit *Aspergillus* growth and aflatoxins production. Screening of antagonistic bacteria against *Aspergillus flavus* in vitro, *Bacillus subtilis* was identified with high antifungal activity.

**Objectives**

*Bacillus licheniformis* has industrial application due to its production of antimicrobial compounds and is related to *B. subtilis* genetically, whose control of competence-sporulation is regulated by a QS mechanism. QS in *B. subtilis* is regulated by ComX pheromone. As QS genes have been identified in *B. licheniformis* NCIMB-8874, our study has focused on QSM in this strain.

**Methods**

To investigate cell-cell communication, the comQX locus was sub-cloned into a shuttle vector which was expressed in *E. coli* and pheromone was isolated by reverse phase chromatography.

**Conclusions**

Pheromone as a QSM is potential signal for communicating between kingdoms and could be applied for bio-control purposes. Identification of new antifungal peptides against *A. flavus* could lead to the development of biotechnological strategies which facilitate control of aflatoxin contamination.

**FEMS-0686**

**Cell-cell communication / Quorum sensing**

## **THE FUNCTIONAL UNITS OF QUORUM SENSING (QS) IN AMMONIA-OXIDIZING BACTERIA**

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### **Background**

Quorum sensing (QS) is an important form of cell-to-cell communication that regulates gene expression in response to fluctuations in cell density. One of the best-studied examples of quorum sensing is *N*-acyl homoserine lactones (AHLs)-mediated cell-cell communication in gram-negative bacteria. While it have been able to describe the QS system in some individual microorganisms for some time now, the functional QS system of nitrifying bacteria has not been completely proved to date.

### **Objectives**

Testing the existence and characterization of quorum-sensing signal produced by nitrifying bacteria (like *Nitrosomonas europaea*, *Nitrosospira multiformis*), and the functional QS signal synthase, the cognate receptor responding to the synthase-generated signal in these microorganisms.

### **Methods**

The AHLs product was obtained by extracting bacteria culture supernatants with acidified ethyl acetate, and then detected by AHLs biosensor and liquid chromatography-mass spectrometry (LC-MS). To identify gene(s) encoding AHL synthases and transcriptional activators, the genome of related nitrifying bacteria was searched for genes with similarity to all QS-related genes. AHL synthases gene was expressed in the heterologous host *E. coli* BL21. Putative AHLs in the recombinant extract were confirmed by comparing the retention times and mass spectra from LC-MS with those of standard AHLs. As a test of the hypothesis that these AHLs were cognate signals of probable receptor, we also examined the solubility of the R protein.

### **Conclusions**



We have shown a LuxI/R type QS signal synthase and regulator in an ammonia-oxidizing strain, and the results provide an opportunity to complete the QS regulatory networks.

**FEMS-0720**

**Cell-cell communication / Quorum sensing**

**ROLES OF QUORUM SENSING-DEPENDENT GLYOXYLATE CYCLE IN  
BURKHOLDERIA GLUMAE**

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**Background**

*Burkholderia glumae*, the causal agent of rice panicle blight, contains a single LuxI-R type quorum sensing (QS) system. TofI synthesizes *N*-octanoyl homoserine lactone (C8-HSL), which is recognized by TofR to regulate various private and public goods. The complex of TofR and C8-HSL activates expression of genes for toxoflavin and flagellar biosynthesis and an IclR type transcriptional regulator gene, *qsmR*. Among QS-dependent public goods, oxalate is produced to protect cells from ammonia-mediated alkaline toxicity in LB during stationary phase. RNAseq analysis indicated that expression of isocitrate lyase gene (*aceA*) might depend on QsmR.

**Objectives**

We aimed to confirm that glyoxylate cycle is controlled by QS and to determine roles of QS-dependent glyoxylate cycle in *B. glumae*.

**Methods**

To ascertain control of glyoxylate cycle by QS, we measured expression level of *aceA* and malate synthase (*glcB*) genes from the chromosomal Tn3-*gusA* fusions in each gene in the wild type and the QS mutants. Electrophoretic mobility shift assays were performed using the promoter regions of *aceA* and *glcB* and purified QsmR. AceA enzyme activity was determined by measuring the formation of glyoxylate-phenylhydrazine in the presence of phenylhydrazine and isocitrate at 324 nm. Levels of oxalate production of each strain were measured by using oxalate assay kit (Libios).

**Conclusions**

Glyoxylate cycle is controlled positively by QS and is important for oxalate biosynthesis in *B. glumae*.

**FEMS-1080**

**Cell-cell communication / Quorum sensing**

**INVOLVEMENT OF RALFURANONE PRODUCTION IN THE VIRULENCE OF  
RALSTONIA SOLANACEARUM OE1-1**

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**Background**

*Ralstonia solanacearum* causes a destructive disease called “bacterial wilt” in numerous plant species. Its virulence is controlled by the transcriptional regulator PhcA, the activity of which is, in turn, regulated in a cell-density dependent manner, termed quorum sensing.

**Objectives**

We herein described the identification and characterization of ralfuranones J–L, new PhcA-regulated secondary metabolites, and the known derivatives, ralfuranones A and B, from *R. solanacearum* strain OE1-1.

**Methods**

Their structures were determined by spectroscopic and chemical methods. These ralfuranones were also detected in vascular exudates from host plants infected with OE1-1. Deletion of *raIA*, which encodes an enzyme for ralfuranone biosynthesis, reduced the virulence of OE1-1 in tomato plants. Virulence was restored by complementation of the *raIA* gene.

**Conclusions**

The results suggest that ralfuranones play important roles in the virulence of OE1-1.

**FEMS-0721**

**Cell-cell communication / Quorum sensing**

**LOSS OF QUORUM SENSING SYSTEM CAUSES OUTER MEMBRANE VESICLE FORMATION IN BURKHOLDERIA GLUMAE**

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**Background**

*Burkholderia glumae* possesses one LuxI-R type quorum sensing (QS) system. The QS system of *B. glumae* activates expression of oxalate biosynthesis genes to avoid ammonia-mediated alkaline toxicity as results of amino acid catabolism. However, QS mutants do not produce oxalate, which causes population crash in stationary phase.

**Objectives**

We aimed to address a question whether QS mutants experience envelop stress to cause vesicle formation.

**Methods**

Outer membrane vesicles (OMVs) were purified from QS mutants using density-gradient sedimentation techniques. Total proteins were identified from the OMVs by LC-MS/MS analyses. The membrane ultrastructure of QS mutants and their vesiculation were visualized by transmission electron microscopy (TEM) after ultrathin sections.

**Conclusions**

Total 368 vesicular proteins were identified from the OMV proteomes. Most vesicular proteins were found to be involved in molecular functions associated with transporter, protein secretion, and protein localization. TEM revealed that periplasmic space of QS mutants is swollen due to hyperhydration in exponential phase. Previous metabolome analyses showed that serious imbalance of glutamate in QS mutants, which suggests that QS mutants might experience a turgor pressure problem. We propose that OMVs of *B. glumae* might be induced to relieve turgor stress caused by imbalanced metabolism.

**FEMS-2407**

**Cell-cell communication / Quorum sensing**

**THE ACTIVATION OF STAPHYLOCOCCUS EPIDERMIDIS SORPTION BY THE AUTOGENOUS LOW MOLECULAR WEIGHT FACTORS**

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**Background:** The bacterial occupation of a surface can be associated with the production and realizing of compounds which can optimize and accelerate this process.

**Methods:** The exponential phase cells of *Staphylococcus epidermidis* strain GISK 33 were washed twice (0.85% NaCl) and diluted to  $10^7$  CFU/ml (PBS, pH 7.2). The cell-free medium filtrates were obtained after the *S.epidermidis* sorption on a polystyrene and glass surface for 60 min. The effect of filtrates media on the bacterial cell adhesion was evaluated. In addition these filtrates were subjected to MALDI-TOF analysis and treatment by trypsin and proteinase K (100 mkg/ml, 37°C, 2 h) with followed by enzymes separation by membrane filtration (10 kDa).

**Conclusions:** The cell-free medium after *S.epidermidis* adhesion to the polystyrene had a stimulating effect on the adhesion of the same bacterial cells on the polystyrene surface compare to the filtrates after the bacterial contacting with the glass surface. The level of found by MALDI-TOF analysis compounds with low mol. weight 552, 574 and 596 Da in the filtrate from polystyrene which showed the greatest stimulatory effect on bacterial adhesion was higher than the glass filtrate. The peptide nature of these compounds was proved by the reduction of the stimulatory effect on the *S.epidermidis* 33 adhesion to the control level (PBS) after the treatment by proteases. Thus, the evidence of the possibility of autoregulation of *S.epidermidis* cell adhesion by using the low molecular weight peptide compounds was provided.

**This work was supported by RFBR (14-04-00687).**

**FEMS-1324**

**Cell-cell communication / Quorum sensing**

**ISOLATION AND PARTIAL CHARACTERIZATION OF A SPORULATION PEPTIDE FROM ENTEROTOXIN-POSITIVE CLOSTRIDIUM PERFRINGENS**

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**Background**

The enterotoxin produced by *Clostridium perfringens* is associated with the sporulation process. The regulation of this process is therefore of interest. We previously described the presence of a low MW, heat-stable compound in the culture fluids of this organism which stimulated the sporulation of homologous and heterologous strains, including enterotoxin-positive strains.

**Objectives**

Isolate and characterize the presumptive peptide involved in promoting sporulation of *Clostridium perfringens*

**Methods**

The peptide was isolated from a defined medium following Sephadex LH-2, SP Sephadex, DEAE Sephadex chromatography followed by HPLC using C8 and C18 columns. Mass spectroscopy, amino acid analysis, and N-terminal sequencing revealed a 1018 Da peptide composed of glutamic acid, glycine, alanine, and an unidentified residue. N-terminal sequencing suggests a branched chain or cyclic structure. The isolate compound was effective in stimulating sporulation of *C. perfringens* in a dose-dependent manner at levels <2 nm/l and differs from sporulation-stimulating peptide previously identified from *Bacillus subtilis* and which was ineffective in promoting sporulation of *C. perfringens*.

**Conclusions**

We believe the peptide functions as a quorum-sensing molecule. The identification of a sporulation signal could help identifying the molecular events leading to sporulation and enterotoxin production by this organism.

**FEMS-2493**

**Cell-cell communication / Quorum sensing**

**QUORUM SENSING INHIBITORY AND ANTI-BIOFILM ACTIVITY OF DIETARY PLANTS CULTIVATED IN KOREA**

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**Background**

The discovery of new agent is demanded by the appearance of multidrug resistant bacteria, like super bacteria, for many years. Also, quorum sensing, cell-to-cell communication, is regarded as remarkable target for development of new antibiotics in recent

**Objectives**

In this study, we investigated antibacterial and anti-quorum sensing (anti-QS) capacity of dietary plants cultivated in Korea and their effect on formation of bacterial biofilms.

**Methods**

Inhibition of quorum sensing (QS) was investigated using the biosensor bacteria *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PA01. The crude extracts of five plants were evaluated on antibacterial activity against five major food pathogen by bi-layer agar well method.

**Conclusions**

Especially, the extract of *Zingiberaceae* plant inhibited production of violet pigment in *C. violaceum* at 10mg/ml and the extracts of *Punica granatum* L. and *Prunella mume* showed anti-QS activity against *C. violaceum*. Biofilm formation was measured by crystal violet and resazurin staining and the extract from *Zingiberaceae* plant had lower minimal inhibitory concentration than that of *Citrus junos* and *Punica granatum* L. All of the extracts showed antibacterial activity against *S. aureus*, *L. monocytogenes*, and *B. cereus*. The extract of *Punica granatum* L. also had high activity against *E. coli* and *S. Enteritidis*. The extract from *Zingiberaceae* plant had lower minimal inhibitory concentration (MIC) than that of *Punica granatum* L. against gram positive bacteria. The extract of *Zingiberaceae* plant had the lowest MIC of 50ug/ml against *S. aureus* and *L. monocytogenes*. These results exhibit the potential of five dietary plants to use as food preservatives and new antibacterial agents.

**FEMS-1379**

**Cell-cell communication / Quorum sensing**

**PSEUDOMONAS FLUORESCENS SBW25 USES TWO SIGNALLING PATHWAYS UTILISING N-ACYL HOMOSERINE LACTON AND ALPHA-HYDROXY KETONES**

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## **Background**

The regulation of biofilm-formation in many pseudomonads is controlled by quorum sensing (QS) and is key to their ability to colonize different environments. However, in the soil and plant-associated *P. fluorescens* SBW25, mutations in diguanylate cyclase-associated genes increase c-di-GMP levels to induce attachment and cellulose expression, resulting in the formation of biofilms at the air-liquid interface of static microcosms, and no involvement of QS-dependent behaviour has been reported.

## **Objectives**

The objective of the study was to examine the ability of SBW25 to quorum sensing.

## **Methods**



Bioinformatics, cultivation assays, TLC and CLSM were used.

## Conclusions

A bioinformatics analysis of the SBW25 genome has identified putative N-acyl homoserine lactone (AHL) and  $\alpha$ -hydroxy ketone (AHK)-dependent QS pathways, including an AHL/AHK synthase-like protein belonging to the HdtS family (PFLU0050) and a CqsA-like protein (PFLU5614). Furthermore, a putative link can be established between QS and the regulation of c-di-GMP levels in SBW25 based on the TpbA/TpbB system of *P. aeruginosa*. In this preliminary work, bioassays with AHL/AHK reporter strains overlaid onto TLC of liquid culture extracts demonstrate that SBW25 produces detectable levels of AHL and AHKs. Although these quorum compounds have yet to be identified, tests using exogenous dodecanoyl homoserine lactone suggest that in SBW25, biofilm structure, eDNA, and possibly siderophore production, may all be regulated by QS pathways. This work is the first to provide experimental proof that SBW25 is capable of responding to AHL/AHK quorum signals like many other pseudomonads, and to suggest that biofilm-formation may also be AHL-regulated under different environmental conditions.

**FEMS-1357**

**Cell-cell communication / Quorum sensing**

**CELL-CELL COMMUNICATION PROVIDES FITNESS BENEFITS TO POPULATIONS OF LISTERIA MONOCYTOGENES IN THE SOIL ENVIRONMENT**

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**Background**

*Listeria monocytogenes* is a ubiquitous opportunistic human pathogen detected in many habitats spanning from the farm environment to food industry and the gastrointestinal tract.

Cell-cell communication participates to the adaptation of bacteria to their environment. In the species *Listeria monocytogenes*, the Agr system is required for full virulence and biofilm formation but its actual role is still poorly understood.

**Objectives**

To investigate whether the ability to communicate provides a benefit to *L. monocytogenes* in soil, a complex environment combining biotic and abiotic characteristics.

**Methods**

Deletion of the gene coding the response regulator or the signal propeptide. Population dynamics of the mutant and/or parental strains in soil microcosms. Both unsterilised and sterilised soils were investigated.

**Conclusions**

Deletion of the gene coding the regulator or the signal did not affect population dynamics in sterilized soil but survival was altered in biotic soil suggesting that the Agr system was involved to face the complex soil biotic environment. This was confirmed by co-incubation experiments. The fitness of the response negative mutant was lower either in the presence or absence of the parental strain but the fitness of the signal negative mutant depended on the strain with which it was coincubated. Survival of the signal negative mutant was higher when cocultured with the parental strain than when cocultured with the response negative mutant. These results showed that the ability to respond to communication provided a benefit to listerial cells. These results might also indicate that in soil, the Agr system controls private goods rather than public goods.



**FEMS-3113**

**Cell-cell communication / Quorum sensing**

**SCREENING OF QUORUM SENSING INHIBITORS WITH AN E. COLI BIOSENSOR AND ENHANCEMENT OF THEIR QUORUM QUENCHING ACTIVITY BY ENCAPSULATING THEM IN CHITOSAN-BASED NANOCAPSULES**

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**Background**

The quorum sensing (QS) hypothesis explains a type of bacterial cell-cell communication which is mediated by means of autoinducer low molecular weight exocellular compounds. This phenomenon has profound implications in the control of many important bacterial traits (e.g. biofilm formation, secretion of virulence factors, etc.). A typical QS system in gram-negative bacterial involves the production and response to acylated homoserine lactone (AHL) signals that are recognized by the LuxR receptor. Several strategies have been conceived to block or disrupt QS (known as quorum quenching, QQ) and thus offer an alternative strategy to reduce the collective power of bacterial pathogens.

**Objectives**

In this regards, we have screened a library of total 24 purified compounds and have tested their QQ activity when applied at a concentration of 1mM.

**Methods**

To this end an *E. coli* Top 10 biosensor reporter of AHL-mediated QS which constitutively expresses green fluorescent protein (GFP) upon exogenous addition of 3-oxo-hexanoyl homoserine lactone (3OC<sub>6</sub>HSL) has been used. A computer-based docking approach was used to elucidate in further detail the interaction between the hit compounds with the TraR protein 3D crystal structure.

**Conclusions**

The hit compounds (i.e. able to inhibit significantly the production of GFP but without inducing a toxic response) were vanillin, trans-cinnamaldehyde, caffeine, genipin, PQS (Pseudomonas quinolone signal) and MOQ (1H-2-methyl-4-quinolone). We have also tested the effect of loading the hit compounds into chitosan-based nanocapsules on their QQ activity and found that this is a strategy to further control and modulate their QQ bioactivity.

FEMS-2706

Cell-cell communication / Quorum sensing

## A LARGE SET OF GENES LINKED TO QUORUM SENSING-DEPENDENT REGULATION IDENTIFIED BY A GENOME WIDE TRANSCRIPTOME ANALYSIS OF SINORHIZOBIUM FREDII NGR234

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### Background

The alphaproteobacterium *Sinorhizobium fredii* NGR234 is outstanding and unique among the rhizobia with its ability to form nitrogen-fixing nodules with a wide range of legumes (1). Beside many other striking features, its 6.9 Mbp genome encodes for two *N*-acyl-homoserine-lactone synthase genes (i.e. *tral* and *ngrl*) which are involved in the biosynthesis of two distinct autoinducer I-type molecules (2,3).

### Objectives

Here we report on the construction of a NGR234- $\Delta$ *tral* and a NGR234- $\Delta$ *ngrl* mutant and their genome wide transcriptome analysis. The high-resolution RNA-seq analysis of early stationary phase cultures in the NGR234- $\Delta$ *tral* background suggested that up to 316 genes were differentially expressed in the NGR234- $\Delta$ *tral* mutant vs. the parent strain and 466 in the background of NGR234- $\Delta$ *ngrl* vs. the parent strain. Accordingly, a common set of 186 genes was regulated by the Tral/R and Ngrl/R regulon including flagella biosynthesis genes and genes linked to EPS succinoglycan biosynthesis. Among the genes that were differentially regulated in NGR234- $\Delta$ *tral* were those linked to replication of the pNGR234a symbiotic plasmid and cytochrome c oxidases. In the NGR234- $\Delta$ *ngrl* mutant biotin and pyrroloquinoline quinone biosynthesis genes were differentially expressed as well as the entire cluster of the NGR234 type III secretion system (T3SS-II). Further we also discovered that genes responsible for rhizopine catabolism in NGR234 were strongly repressed in the presence of high levels of *N*-acyl-homoserine-lactones.

### Methods

high-resolution RNA-seq analysis

### Conclusions

Together with nodulation assays, our RNA-seq-based findings suggested that QS-dependent gene regulation appears to be of higher relevance during non-symbiotic growth rather than for life within root nodules.

**FEMS-2645**

**Cell-cell communication / Quorum sensing**

**REGULATORY RNA ARISES FROM 3'-UTR OF AHL SENSOR ENCODED RNA AFTER ITS PREPROCESSING IN PECTOBACTERIUM ATROSEPTICUM**

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**Background**

In a broad group of proteobacteria two quorum sensing-related genes, which encode synthase of autoinducers and their sensor, respectively, have convergent topology and overlap by their 3'-ends. It was suggested that the expression of one gene may antagonize the transcription of another one because of the convergent arrangement of these genes.

**Objectives**

We proposed that the topology of quorum sensing-related genes of plant pathogenic bacterium *Pectobacterium atrosepticum*, *expI* and *expR*, determines the mechanism of regulation of their expression.

**Methods**

We noticed that *expR* gene lacks any obvious transcriptional stop signals. Using chain-specific RT-qPCR we found that at the stationary growth phase *expR* mRNAs having long 3'-untranslated region (UTR) were formed and the expression of oppositely oriented *expI* gene was simultaneously decreased. It is likely, that RNA products of the *expR* regulatory gene may inhibit the expression of the autoinducer synthase gene through the interactions with *expI* mRNA. Using 3'-RACE method we found that at the stationary growth phase the extended *expR* transcripts underwent preprocessing resulting in their break down into two fragments. The first fragment encompassed the entire length of open reading frame (ORF) of *expR* gene and the second one included only a long 3'-UTR having a predicted regulatory function.

**Conclusions**

We found that *cis*-encoded RNA may participate in the regulation of expression of two convergent functionally related genes. The length of one of two transcripts varies respective to the cell growth phase affecting the expression of oppositely oriented gene. Moreover, *cis*-encoded regulatory RNAs arises from protein encoded RNAs that undergo preprocessing in their 3'-UTR.

FEMS-1727

Cell-cell communication / Quorum sensing

## PROTEOME PROFILING OF BURKHOLDERIA PSEUDOMALLEI QUORUM SENSING MUTANTS

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### Background

*Burkholderia pseudomallei*, the causative agent of melioidosis, is a gram-negative soil bacterium in tropical areas. *B. pseudomallei* employs several *N*-acyl-homoserine lactone (AHL)-mediated quorum sensing (QS) systems, which activate specific sets of genes as a function of cell density [1]. The genome of *B. pseudomallei* encodes genes for three QS systems with one *luxI* and one *luxR* homologue, respectively, and additionally three orphan *luxR* homologues [2]. The *luxI* homologues encode AHL synthases, which produce specific AHLs binding to the respective transcriptional regulator and thus regulating expression of specific genes involved in virulence like biofilm formation, siderophore biosynthesis or swarming motility [3].

### Objectives

The aim was to clarify the influence of the different QS systems on virulence factor expression.

### Methods

We constructed mutants in the synthase-coding genes. Subproteome fractions of the mutant strains were subjected to mass spectrometry analysis to identify targets of the different QS systems. We used the DIA approach IMS<sup>E</sup> in combination with the Hi3 approach for quantification of cytosolic proteins and the GeLC MS/MS approach for the analysis of extracellular proteins. Furthermore, the AHL spectrum, synthesized by the different QS systems was determined by analytical thin layer chromatography.

### Conclusions

The *luxI* homologues influence protein expression by up- and downregulation of AHL-dependent proteins in the QS circuitry of *B. pseudomallei*.

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**FEMS-1259**

**Food biotechnology**

## **ENZYMATIC PRODUCTION OF MODIFIED STARCHES WITH SLOW DIGESTION PROPERTIES**

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### **Background**

In human nutrition, the proper rate of glucose release and absorption from digesting starch may play important roles in body health. Therefore, improving food quality with higher amounts of slow digestible starch is becoming an area of interest for researchers in academia and industry.

### **Objectives**

The objectives of this study is to produce the modified starch with slow digestion properties by starch-modifying enzymes.

### **Methods**

Two branching enzymes (BEs), PH1386 of *Pyrococcus horikoshii* OT3 and Athe\_0558 of *Caldicellulosiruptor bescii*, were expressed in *E. coli* and purified by Ni-NTA affinity chromatography. The enzymatic properties of two BEs have been studied by high performance anionic exchange chromatography (HPAEC) analysis. The melting temperature range (Tr) and melting enthalpy ( $\Delta H$ ) representing the degree of retrogradation of modified starches were also studied by differential scanning calorimetry. Finally, the digestion properties of modified starches were examined by pancreatic enzyme and  $\alpha$ -glucosidase.

### **Conclusions**

HPAEC analysis of BE-modified starches revealed that PH1386 produces the branch chains with mainly DP 10-12, while Athe\_0558 prefers DP 6-7. The proportion of A chains (DP  $\leq 12$ ) of amylopectin was increased and the proportion of B1 (DP 13-36) and B2 chains (DP  $\geq 37$ ) was decreased compared to control. The Tr and  $\Delta H$  also decreased compared to control, suggesting that retrogradation rate of the BE-modified starches was delayed. The increased resistant starch contents and

decreased rapidly digestible starch contents of BE-modified starches are thought to be due to the increase in  $\alpha$ -1,6 linkages and the highly branched structure of starch.

**FEMS-1788**

**Food biotechnology**

**MAXIMIZING BIOTECHNOLOGICAL PRODUCTION OF 3-HYDROXYPROPIONALDEHYDE AS A NATURAL FOOD PRESERVATIVE WHILE MINIMIZING ITS CONVERSION TO TOXIC ACROLEIN**

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**Background**

3-hydroxypropionaldehyde (3-HPA) has strong antimicrobial activity and application potential as a natural food preservative for disinfection of minimally processed vegetables. During glycerol metabolism, *Lactobacillus reuteri* DSM 20016T can accumulate 3-HPA, which undergoes reversible dimerization and hydration, and can be further converted enzymatically into inactive 1,3-propanediol (1,3-PD), restoring intracellular redox potential. Dehydration of 3-HPA resulting in acrolein, a toxic metabolite, is also possible, however, conditions promoting this transformation have not been fully understood due to the lack of analytical methods to simultaneously quantify 3-HPA as well as acrolein.

**Objectives**

The aim of this study was to maximize the capacity for biotechnological processing of glycerol to 3-HPA using *L. reuteri*, while accounting for the accumulation of acrolein after production and storage to evaluate toxicological risk.

**Methods**

Strategies to improve 3-HPA production included the addition of redox-active compounds during glycerol fermentation to prevent conversion to 1,3-PD, the usage of immobilized cells resulting in high densities of viable cells as well as the optimization of process parameters in batch and fed-batch processes. Special attention was paid to cultivation conditions of *L. reuteri* prior to glycerol conversion. Acrolein and 3-HPA were simultaneously quantified using a newly established analytical method employing ion-chromatography with pulsed amperometric detection.

**Conclusions**

Redox-active compounds, process conditions and pre-treatment of *L. reuteri* cultures influenced 3-HPA yield as well as ratio of 3-HPA to 1,3-PD. Temperature and pH

optimization prevented formation of acrolein during biotechnological production and storage, promoting the safety of 3-HPA when applied as natural food preservative.

**FEMS-2626**

**Food biotechnology**

**COMPARATIVE STUDY OF FERMENTATION PROCESSES BY FREE AND IMMOBILIZED GRAPEVINE INDIGENOUS STRAINS FOR THE PRODUCTION OF HIGH QUALITY WINES**

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**Background**

Vinification is a complex microbiological process. Immobilized microorganisms constitute an alternative approach aiming to lower the overall cost of production and improve wine quality.

**Objectives**

This study focuses on the immobilization of indigenous *Saccharomyces* and non-*Saccharomyces* strains for use in wine production.

**Methods**

*Saccharomyces cerevisiae* strains are known for their ability to produce high ethanol levels, while non-*Saccharomyces* species contribute to the enhancement of the wine flavor resulting in high quality wines. Two indigenous environmental yeast strains isolated from a Greek agricultural region (Zitsa, Epirus), *Saccharomyces cerevisiae* Z622 and *Metschnikowia pulcherrima* var Zitsae ZY6 were used as starter cultures in a sequential fashion for wine fermentation. Delignified cellulose is used as an immobilization support meeting all requirements and demands for use in food production and wine making.

**Conclusions**

Both yeast strains were successfully immobilized maintaining their metabolic activity during fermentation. Immobilized cells showed a better overall metabolic activity compared to control free cells.

**Acknowledgments**

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**FEMS-2285**

**Food biotechnology**

**LACTOBACILLUS SAKEI ISOLATED FROM FERMENTED MEAT: EXTERNAL ANTIBACTERIAL ACTIVITY AGAINST LACTIC ACID BACTERIA AND PATHOGENIC STRAINS**

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**Background**

Lactobacilli play an important role in the production of fermented sausages, due to their antimicrobial activity and their effect on the organoleptic properties of meat products. *Lb. sakei* is gaining relevance because it produces bacteriocins with anti-listerial activity. However, the use of bacteriocins may be limited due to their action against phylogenetically related species and ineffectiveness against some pathogenic and undesirable spoilage bacteria. Another group of proteins with antibacterial activity are peptidoglycan hydrolases, which so far have not been reported for this bacterial species and probably play a role in food safety and in the organoleptic properties of meat products; because they prevent the growth of pathogens and spoilage bacteria, including heterofermentative lactic acid bacteria (LAB).

**Objectives**

The aims of this work were to identify lactic acid bacteria isolated from fermented meat, as well as the proteins responsible for their extracellular antibacterial activity against different pathogenic and LAB strains

**Methods**

Isolated lactobacilli were identified by 16S rDNA sequencing. Antimicrobial and lytic activities were evaluated in the culture supernatant by agar diffusion tests and zymography, using in both cases different target microorganisms. A 79-kDa protein was identified which showed antimicrobial activity. Protein identification was performed by LC/MALDI TOF/TOF and the data showed a high correspondence with a putative N-acetylmuramoyl-L-alanine amidase.

**Conclusions**

A *Lb. sakei* strain isolated from fermented meat which has displayed external antibacterial activity has been cultured and studied for the first time. The aforesaid activity is probably related to an protein with peptidoglycan hydrolase activity. This may have a potential use in food preservation, considering that this activity was obtained from a bacteria isolated from a meat product.

**FEMS-2437**

**Food biotechnology**

**LEVANSUCRASE OF PSEUDOMONAS SYRINGAE AS PRODUCER OF NOVEL PREBIOTIC FRUCTANS: A STRUCTURE-FUNCTION STUDY OF THE ENZYME**

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**Background**

Gut microbiota has numerous roles in human life and its significance has thus far been underestimated. Therefore, more detailed studies on diversity and metabolism of gut microbiota are needed.

**Objectives**

Gut microbiota can be shaped by prebiotics. Levan-type ( $\beta$  2,6-linked) fructans can be considered as potential prebiotics<sup>[1]</sup>. We have synthesized levan and levan-type fructooligosaccharides by heterologously expressed levansucrase Lsc3 of *Pseudomonas syringae* pv. tomato. These substrates should affect gut microbiota as were perfectly fermented by a gut generalist *B. thetaiotaomicron*<sup>[2]</sup>. Here we focus on structure-function relationships of the Lsc3 protein and its biotechnological potential.

**Methods**

The His-tagged Lsc3 protein was site-directedly mutated, biochemical methods were used to characterize the mutant proteins and 3D structure modelling was applied to interpret the results.

**Conclusions**

Levansucrase Lsc3 has high polymerizing activity (up to 80%) and extremely high stability confirming its biotechnological potential for the synthesis of fructans. Several novel catalysis-related positions for levansucrases were revealed and their predicted location on a 3D model of Lsc3 will be presented.

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**FEMS-3150**

**Food biotechnology**

**MATCHING GENOME AND TRANSCRIPTOME OF LACTOCOCCUS LACTIS STRAINS WITH ROBUSTNESS TOWARDS INDUSTRIAL STRESSES**

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**Background**

*Lactococcus lactis* is industrially employed to manufacture fermented dairy products. Spray drying is the cheapest preservation method for *L. lactis* starter cultures, but during this process cultures encounter heat and oxidative stress, resulting in low survival. Viability of starter cultures is essential for their role in dairy acidification, supporting the need to understand and improve their robustness.

**Objectives**

We have measured the ability of 39 *L. lactis* strains to survive industrially relevant stresses. This set of strains contained different *L. lactis* strains from dairy as well as plant origin. The observed 4-log variation in heat and oxidative stress survival was compared with genomic content, resulting in the identification of genes associated with robustness. Presence and activity of genes can play an important role in the observed diversity in robustness.

**Methods**

Therefore, four *L. lactis* strains with varying robustness phenotypes were fermented under twelve different conditions, varying in temperature, salt concentration, pH, and oxygen level. Cells were harvested at exponential phase of growth for transcriptome analysis and survival measurements.

**Conclusions**

The varying growth conditions resulted in up to 4-log differences in robustness towards heat and oxidative stress. Moreover, clear differences in gene expression profiles were observed. Correlation of robustness phenotypes and gene expression levels revealed transcriptome signatures for oxidative and heat stress survival. For strain MG1363 this included the *metC-cysK* operon, involved in methionine and cysteine metabolism, which triggered us to grow this strain in the absence of

cysteine, resulting in elevated expression levels of the *metC-cysK* operon and concomitant enhanced robustness towards oxidative stress.

**FEMS-2919**

**Food biotechnology**

## **IMPROVEMENT OF SECRETORY EXPRESSION FOR THE SWEET-TASTING PROTEIN BRAZZEIN IN YEAST**

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### **Background**

Brazzein is an intensely sweet-tasting protein with four disulfide bridges. In previous study, we constructed the secretory expression system of recombinant brazzein in yeast *Kluyveromyces lactis*.

### **Objectives**

In this expression system, the control of protein disulfide-bond formation in the endoplasmic reticulum (ER) is often a bottleneck for secretory protein production. The major pathway for protein disulfide-bond formation in endoplasmic reticulum (ER) includes the conserved ER-membrane protein Ero1p and protein disulfide isomerase (PDI).

### **Methods**

In the present study, the PDI gene was introduced in the yeast *Kluyveromyces lactis* to improve the secretory efficiency of brazzein. The expression of Ero1p was also induced by treatment of the cells with dithiothreitol. The culture condition of the yeast transformants for high yield secretion of the recombinant des-pE1M-brazzein was in YPGal medium for 96 hours at 30 °C.

### **Conclusions**

The amount of misfolded or unfold recombinant des-pE1M-brazzein remaining inside the cell decreased and the amount of the secretory recombinant des-pE1M-brazzein having an intrinsic sweetness increased approximately 1.7-fold than that of the previous expression system. These results demonstrate that the modified *K. lactis* expression system could be applicable to mass production of the recombinant brazzein with attributes useful in the food industry.

**FEMS-2181**

**Food biotechnology**

**IDENTIFICATION OF SULPHITE RESISTANCE WINE YEASTS  
SACCHAROMYCES CEREVISIAE FROM A POOL OF SELECTED STRAINS**

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**Background**

Sulphite is frequently added in winemaking, hence sulphite resistance mechanisms have been studied extensively in *Saccharomyces cerevisiae*. Research turned out that yeasts possess plasma membrane protein encoded by the *SSU1* gene (lies on chromosome XVI), which excretes sulphite from yeast cells. Some highly sulphite-resistance wine strains have an *SSU1* allele – *SSU1-R* - on chromosome VIII. We tried to elucidate, how long adaptation to high sulphite concentrations affects to the evolution of yeast strains.

**Objectives**

The aim of experiment was to determine presence of *SSU1* and *SSU1-R* genes and their expression in selected *Saccharomyces cerevisiae* strains.

**Methods**

After 10 years of storage with SO<sub>2</sub> (concentration ~ 400 mg/ml) the pool of 12 strains *S. cerevisiae*, include 11 sulphite strains and negative control strain, were tested for the presence of the *SSU1* or *SSU1-R* gene. We analysed them by using PCR assays, gel electrophoresis and DNA sequencing. Obtained sequences were aligned, in CLUSTAL W tool, with known sequences from databases (BLAST and SGD). Gene expression was tested during alcoholic fermentation for few strains by real-time PCR.

**Conclusions**

The presence of the *SSU1* gene was confirmed in 7 strains (include control strain) and *SSU1-R* gene in 3 strains. None of those genes was detected in 2 strains. The results indicate that possession of the *SSU1* or its allele is independent of the long term storage in must with SO<sub>2</sub>. It is well-known that evolution in yeast cells occur dynamic. In our research it was confirmed by the presence of *SSU1* in negative control strain.

**FEMS-0704**

**Food biotechnology**

## **INFLUENCES OF DIFFERENT MINERALS ON THE GROWTH AND CITRIC ACID PRODUCTION OF *YARROWIA LIPOLYTICA***

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### **Background:**

Citric acid is the most important and widely used organic acid produced by fermentation. Mineral composition of the fermentation medium has a critical role in citric acid production.

### **Objectives:**

The aim of this study was to investigate the influences of different minerals on growth and citric acid production of *Yarrowia lipolytica* NBRC 1658 in a glucose medium.

### **Methods:**

The mineral salts; FeSO<sub>4</sub>, CuSO<sub>4</sub>, MnSO<sub>4</sub> and ZnSO<sub>4</sub> were added to the fermentation medium separately with concentration ranges of 0.01-0.1, 0.001-0.02, 0.005-0.05 and 0.002-0.008 g/L, respectively. Experiments were performed in a batch system. Biomass concentration was determined spectrophotometrically. Concentration of citric acid was measured by pyridine-acetic anhydride method.

### **Conclusions:**

In this study it was obtained that by the addition of FeSO<sub>4</sub> into the media, the specific growth rate of the yeast increased and although CuSO<sub>4</sub> addition had a positive effect on the decrease of the duration of the lag phase, it had an adverse effect on the citric acid production as well. The maximum specific growth rate of the yeast determined as 0.053 h<sup>-1</sup> by using 0.02 g/L of MnSO<sub>4</sub> in the glucose based medium. Although ZnSO<sub>4</sub> supported the growth of the yeast, citric acid concentration reduced and reached to a maximum value of 13.4 g/L. It was also concluded that citric acid production decreased by the supplement of the salts examined within the concentration ranges above, while certain concentrations of FeSO<sub>4</sub>, MnSO<sub>4</sub> and ZnSO<sub>4</sub> had profound effects on the growth of the yeast.

**FEMS-2831**

**Food biotechnology**

## **ENHANCEMENT OF THE BIOAVAILABILITY OF EXTRACTABLE BERRY PHENOLICS BY SOLID STATE FERMENTATION**

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### **Background**

Increasing interest can now be observed for the utilization of plant-wastes rich in extractable phenols derived from fruit processing. Using these residues, free phenolics with high antioxidant potential can be liberated by solid-state fermentation, via the deglycosylation action of beta-glucosidases of the fermenting fungi. Thus, the bioavailability of the extractable phenols can be enhanced since aglycons get across the cell membrane easily.

### **Objectives**

In our previous studies, isolates of *Rhizomucor miehei* and *Mucor corticolus* showed intensive extracellular beta-glucosidase activity on plant-derived residues. Here, we analyzed the ability of these zygomycetes to generate free phenolics under jostaberry-based solid-state fermentation.

### **Methods**

Solid pomace medium was supplemented with soy flour as nitrogen source. During incubation samples were taken every second or third day and extracted with distilled water, ethanol:water 50:50 or HCl:methanol 10:90 solutions. Besides beta-glucosidase activity, total phenolics and total anthocyanins were determined. Antioxidant activity tests including radical scavenging capacity and ferric reducing capacity assays were also performed.

### **Conclusions**

The *R. miehei* and *M. corticolus* bioconversion of jostaberry pomace leads to enhanced total phenolic content. Positive correlation between total phenolic content and beta-glucosidase activity was found in both systems. Ferric reducing antioxidant power was slightly increased during the first phase of fermentation; however, anthocyanin content showed significant drop after 3-5 days. Results indicate that *R. miehei* and *M. corticolus* fermentations are applicable methods for liberation and enrichment of health-relevant extractable phenolics from jostaberry pomace. This research was supported by the Hungarian Research Fund (OTKA PD 112234).

**FEMS-2158**

**Food biotechnology**

**INFLUENCE OF STARTER INOCULUM ON DYNAMIC GROWTH OF  
STREPTOVERTICILLIUM MOBARAENSE IN O3 MEDIUM.**

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**Background**

*Streptoverticillium mobareense* KKP 2013 strain obtained from the Culture Collection of Industrial Microorganisms is Gram-positive *Actinomyces* commonly occurring in natural environment. Under certain conditions, it has the ability to produce the enzyme MTG, protein-glutamine gamma-glutamyltransferase, EC 2.32.13, commonly called transglutaminase. Transglutaminase catalyzes the formation of a covalent bond between the proteins, it allows also crosslinking reactions, namely the production of new intra- or intermolecular bonds between the proteins and peptides. MTG due to its characteristics is widely used in the production of meats, dairy and bakery products.

**Objectives**

In the process for producing transglutaminase one of the important steps is to obtain an appropriate number of microorganisms, which allows to obtain the highest possible quantity of active enzyme.

**Methods**

In our study, we tried to determine the growth rate of strain KKP 2013 *S. mobaraense* in the proliferating medium O3. In the precise number of microorganisms on the breeding stage, we used the method of pour plate technique on PCA medium, according to PN-EN ISO 4833-1: 2013-2014.

**Conclusions**

We established the optimal volume of medium in relation to the number of bacteria in the starting inoculum, which will produce the highest activity of the resulting enzyme.

**FEMS-2779**

**Food microbiology**

**USING DYNAMIC SINGLE CELL BIOLOGY TO DISSECT INJURY,  
RESUSCITATION AND DEATH PHENOMENA IN STRESSED POPULATIONS OF  
ESCHERICHIA COLI**

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**Background**

While inimical food preservation approaches often result in heavily damaged and stressed populations of foodborne pathogens and spoilage microorganisms, very little is known about the actual molecular and genetic events and dynamics that govern the (sub)lethal injury and subsequent resuscitation or death of these cells. Nevertheless, insights into these phenomena might be decisive for a proper understanding of the resulting behavior and evolvability of the surviving subpopulation.

**Objectives**

In this study, we set out to monitor and dissect (sub)lethal injury and resuscitation phenomena in populations and cells of *Escherichia coli* that are stressed by heat or high hydrostatic pressure treatment.

**Methods**

Directed evolution, genetics and time-lapse fluorescence microscopy.

**Conclusions**

Based on adaptive mutations, genetics and live cell biology, different subcellular processes and structures have been delineated and monitored that play a role in the heterogeneous injury and resuscitation dynamics of high temperature or pressure stressed *E. coli* cells and populations, thereby revealing a surprising impact on the spatial dynamics of nucleoids and protein aggregation inside stressed cells.



**FEMS-0566**

**Food microbiology**

**INHIBITION OF *S. AUREUS* A, B, C AND D ENTEROTOXINS BY SUBINHIBITORY DOSES OF TERPENOID PHENOLS FROM PLANTS ESSENTIAL OILS**

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**Background**

*Staphylococcus aureus* is a pathogen of major concern for clinical infection and foodborne illness and has remarkable invasiveness and toxin-mediated virulence. Studies have shown that plant essential oils can influence production of toxins, avoiding overuse of antibiotics and the development of bacterial resistance.

**Objectives**

This study aimed to verify the influence of major terpenoid phenols compounds from plants essential oils in subinhibitory doses on enterotoxins A, B, C and D produced by *Staphylococcus aureus*.

**Methods**

Five phenolic compounds (eugenol, geraniol, cinnamaldehyde, citronellol and terpineol) were tested. Amounts corresponding to 60% and 80% of Minimum Inhibitory Concentration (MIC<sub>90%</sub>) were added to culture medium Tryptic Soy Broth (TSB). Each treatment was inoculated with a suspension of *S. aureus* producer of each enterotoxin A, B, C and D and incubated (37°C/24h). TSB without compounds was used as positive control. After incubation each treatment was centrifuged (9000g/4°C/30min) and supernatant was used to detect enterotoxin production by Reverse Passive Latex Agglutination. Interpretation of results was performed according to manufacturer's instructions kit. Agglutination was characterized as: strong (+++); moderate (++); weak (+) and absence (-) of enterotoxin production. Assays were performed in duplicate. Kruskal-Wallis nonparametric test was performed using scores related to enterotoxin production.

**Conclusions**

Some phenolic compounds from plants essential oils tested demonstrated great capacity to inhibit enterotoxins produced by *S. aureus* and, consequently decreasing its virulence. Therefore, it may be a promising way to prevent diseases caused by enterotoxigenic *S. aureus* and to contribute for reduction of antimicrobial drugs consumption.



**FEMS-1947**

**Food microbiology**

## **CHARACTERIZATION OF THE DAIRY STREPTOCOCCUS THERMOPHILUS ACA-DC 29 STRAIN THROUGH COMPARATIVE GENOMICS**

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### **Background**

Although the *Streptococcus* genus includes mainly pathogenic species, *Streptococcus thermophilus* is a widely used dairy starter culture of great economic importance for the food industry. *S. thermophilus* has been adapted to milk probably through a degenerative evolution process that has led to the loss of typical streptococcal pathogenic traits.

### **Objectives**

The genome sequence of the yogurt isolate *S. thermophilus* ACA-DC 29 was analyzed for assessing the technological potential of this strain. Comparative genomics analysis was also performed between the genome of ACA-DC 29 and the existing complete genome sequences of *S. thermophilus*.

### **Methods**

The genome sequence of ACA-DC 29 was annotated using online annotation tools. Full chromosome alignments were calculated with Progressive Mauve. The pangenome, the core genome and the unique genes were predicted with the GView Server. The genomic islands, the CRISPRs and the antimicrobial peptides were predicted with IslandViewer, CRISPRcompar and BAGEL3, respectively.

### **Conclusions**

The analysis of the *S. thermophilus* ACA-DC 29 genome sequence revealed the absence of pathogenic features. Genes related to the adaptation to milk were identified. Full chromosome alignments showed a high degree of synteny among the different strains. The pangenome of the eight strains comprised of approximately 2,300 genes. Concerning the ACA-DC 29 strain, approximately 250 unique genes involved in various biological processes were also identified. Further analysis indicated that several of them may have been acquired through horizontal gene transfer. Five potential antimicrobial peptides and two CRISPR systems, which may confer resistance against phages, were also predicted.

**FEMS-1972**

**Food microbiology**

## **GREEK TRADITIONAL DAIRY AND MEAT PRODUCTS: A BIOLOGICAL RESERVOIR FOR NEW PROBIOTIC STRAINS**

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### **Background**

Probiotics provide health benefits to the host upon ingestion. Lactic acid bacteria are frequently used as probiotics. Important qualifying characteristics of probiotics strains are the acid and bile resistance, the absence of pathogenic traits, the elevated adhesion ability, the production of antimicrobial substances and the inhibition of pathogens. Traditional fermented foods constitute a promising source for discovering new probiotic strains.

### **Objectives**

Lactic acid bacteria isolated from Greek traditional dairy (Yogurt and Feta, Kasseri, Xynotyri, Graviera, Kopanisti, Formaella, Galotyri, Kefalotyri cheeses) and meat (Lountza and Salami) products, were screened for their probiotic potential.

### **Methods**

The isolated strains were evaluated for survival to low pH and in the presence of bile salts. The safety of selected strains was assessed by studying their haemolytic activity and their susceptibility to commonly used antibiotics. Antimicrobial activity against pathogens, was also examined. Finally, the ability of the strains to adhere to collagen-coated 96-well microplates and to the human colorectal adenocarcinoma cell lines HT-29 and Caco-2 *in vitro*, was investigated.

### **Conclusions**

Of the 128 isolated strains, thirty were selected for further study due to their robust performance under the adverse conditions of the gastrointestinal tract. None of the strains exhibited haemolytic activity while variable antibiotic resistance was detected. Two *Streptococcus thermophilus* strains presented inhibitory activity against *Streptococcus anginosus* LMG 14502T and *Streptococcus mutans* LMG 14558T. Three *Lactobacillus plantarum* strains were found to adhere better to both cell lines,

as well as to the collagen-coated microplates. The utilization of *in vitro* tests enabled the selection of strains with promising probiotic features.

**FEMS-1872**

**Food microbiology**

## **NATIONWIDE OUTBREAK OF LISTERIOSIS DUE TO READY-TO-EAT SALAD IN SWITZERLAND**

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### **Background**

*Listeria monocytogenes* is an important foodborne pathogen with a significant impact on public health and economy worldwide. Human infections are rare, but *L. monocytogenes* has the potential to cause serious and life-threatening disease. Clinical conditions primarily comprise septicemia, meningitis, meningoencephalitis and abortion.

### **Objectives**

To describe a nationwide outbreak of listeriosis having occurred during 2013 to 2014 in Switzerland.

### **Methods**

Microbiological methods (serotyping, MLST, PFGE) and epidemiological investigations by telephone interviews were used.

### **Conclusions**

From 26 October 2013 to 23 April 2014, 32 cases of listeriosis infected with an *L. monocytogenes* strain serovar 4b, sequence type 4 and belonging to a single distinct PFGE pulsotype were registered in patients from several cantons of Switzerland. *L. monocytogenes* was detected in blood (75%), CSF (16%), ascites (6%) and in joint fluid (3%) samples. By the end of March 2014, a retail company reported an *L. monocytogenes* contamination of ready-to-eat salads to the authorities after detecting the pathogen through its in-house routine quality control. Product and environmental samples collected during subsequent investigations yielded isolates, matching the outbreak strain, thus confirming that ready-to-eat salad from this company was most likely the outbreak source. The cause for the product contamination was related to a design-inherent hygienic problem of one specific product-feeding belt. Complementary patient interviews also identified ready-to-eat green salads bought at one retailer as the likely outbreak source.

**FEMS-0604**

**Food microbiology**

## **RAPID AND ACCURATE LISTERIA SPP. IDENTIFICATION BY MALDI BIOTYPER DATABASE IMPROVEMENT**

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### **Background**

*Listeria* species are closely related to each other, however, only *L. monocytogenes* and *L. ivanovii* exhibit pathogenic features, (human cases of *L. ivanovii* infection being quite rare). Rapid, accurate identification of *Listeria* strains is essential for appropriate management and timely intervention for infection control.

The accuracy and speed of data acquisition by MALDI-TOF MS makes this an important tool for biological public health hazards, food processing, quality control, and disease diagnoses.

### **Objectives**

Since *Listeria* species are closely related and have similar profile spectra, the objective was to reliably identify the different *Listeria* species by MALDI-TOF MS through establishment of a reliable library of mass spectral *Listeria* fingerprints from reference strains. Therefore the database was enhanced by 52 *Listeria* strain references, from 9 species.

### **Methods**

*Listeria* strains were grown on Columbia Agar with 5 % Sheep Blood agar (BD, Heidelberg, Germany) at 37 °C. Biomass was collected after one day, and then processed by ethanol / formic acid standard extraction method. Mass spectra were acquired by using microflex LT (Bruker Daltonik, Bremen, Germany) and identified with MALDI Biotyper Compass software.

### **Conclusions**

With the new, optimized references database, it was possible to identify all tested *Listeria* strains from 9 species. This was proven by analysis of more than 1200 mass spectra.

Although log (score) value differences were small between *Listeria* species, the correct species was always the best match, no false identification occurred. In consequence, the important identification of the pathogenic *Listeria* species is possible with easy, rapid and reliable low-cost MALDI-TOF MS method.

**FEMS-1468**

**Food microbiology**

**INCIDENCE OF ASPERGILLUS PRODUCER OF OCHRATOXIN A IN THE TROPICAL VITICULTURE IN THE NORTHEAST OF BRAZIL**

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**Background**

The biodiversity of filamentous fungi is one of the most important contributions to the occurrence of mycotoxins in agricultural products. The species belonging to the genus *Aspergillus* has been blamed for the presence of mycotoxins in wines. The incidence of these genera in grapes will depend on climatic conditions, grape variety, cultivation form and agricultural practices.

**Objectives**

This study aimed to identify *Aspergillus* species isolated from wine grapes at harvest, from organic and conventional vineyards, in the São Francisco Valley.

**Methods**

Varieties evaluated were Touriga Nacional and Ruby Cabernet, and Tempranillo. For the isolation of fungi direct plating was carried out in DRBC culture medium, 25°C/7 days. The isolates were identified using standard culture media. The determination of toxigenic potential was performed by Thin Layer Chromatography.

**Conclusions**

There was no presence of *Aspergillus* in the Tempranillo. All *A. carbonarius* were producers of ochratoxin A. The presence of these species can spoil the grapes. All species identified are naturally present in the vineyards.



**FEMS-1472**

**Food microbiology**

**INCIDENCE OF OCHRATOXIGENIC SPECIES OF ASPERGILLUS AND OCHARATOXIN A ON COFFEE BEANS FROM CONVENTIONAL AND ORGANIC CULTIVATION**

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**Background**

The natural microbiological contamination of fruit and coffee beans, are due to the presence of bacteria, yeasts and filamentous fungi, including fungi species of the genus *Aspergillus* ochratoxin-producing (OTA) has great relevance for coffee security.

**Objectives**

The objective of this study was to identify ochratoxigenics fungi and quantify the presence of OTA in conventional and organic cultivation of coffee beans in the southern region of Minas Gerais (Brazil) and compare the concentrations in both systems.

**Methods**

We analyzed 30 samples of coffee beans (*Coffea arabica* L.), 20 samples of conventional coffee and 10 organic coffee samples. The incidence of OTA in the samples of coffee beans was performed by high performance liquid chromatography (HPLC).

**Conclusions**

Samples were identified 480 fungi of the genus *Aspergillus* Section *Nigri* and *Circumdati*. The main producing species *Aspergillus ochraceus* was OTA (83% of the samples). For the OTA in the grain, only one sample collected organic coffee ground, presented OTA, 1.12 ug / kg. Considering these results, it can be stated that the presence of *Aspergillus ochraceus* OTA producer does not imply contamination of grain by mycotoxins and two coffee cultivation systems have the same risk of contamination.

**FEMS-0898**

**Food microbiology**

**RATIONAL SELECTION OF YEASTS BASED ON THEIR PECTINOLYTIC ACTIVITIES AND ITS INCIDENCE ON TECHNOLOGICAL AND SENSORIAL ASPECTS OF WINE QUALITY**

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**Background**

Pectinase enzymes have shown a considerable influence in both, sensitive and technological properties of wines. They can help to improve clarification and filterability process, releasing more color and flavor compounds entrapped in the grape skin and facilitating the liberation of phenolic compounds. The addition of commercial enzyme preparations, with filamentous fungi as its main source, can be quite expensive for industry.

**Objectives**

This work aims to find yeasts that, because of their native pectinases, can be applied on combined fermentations with *Saccharomyces cerevisiae* obtaining significant benefits over traditional wine fermentations.

**Methods**

785 yeast strains isolated from wineries were identified and tested for several enzymatic activities of recognized interest for enology industry. The impact of *Metschnikowia pulcherrima* as a source of pectinolytic enzymes during wine fermentation was analyzed by measuring its influence on filterability, turbidity and the increase on color, anthocyanin and polyphenol content of wines fermented in combination with *Saccharomyces cerevisiae*. Further metabolites with enological interest were analyzed during the entire fermentation period.

**Conclusions**

Positive results were obtained in all expected parameters when *M. pulcherrima* was used by comparing wines fermented with *S. cerevisiae* alone and combined with *Kluyveromyces thermotolerans*, even working better than commercial enzymes preparations in most parameters. Additionally, *M. pulcherrima* selected strain was used in a semi-industrial scale combined with three different *S. cerevisiae* strains, obtaining meaningful results on sensorial parameters like total polyphenol index, color intensity and turbidity of wines and on technological properties as wine filterability.

**FEMS-1627**

**Food microbiology**

**DETECTION AND TYPING OF LACTOBACILLUS PARABUCHNERI, A  
POTENTIAL SPOILAGE ORGANISM, IN MILK AND CHEESE**

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**Background**

Histamine can be formed by microorganisms in fermented products such as cheese. High concentrations of this compound can trigger symptoms in consumers who have a histamine intolerance. Microbial decarboxylation of histidine is probably the major cause of histamine formation in cheese. We isolated histamine-producing bacteria from various raw-milk cheeses containing histamine levels higher than 50 mg kg<sup>-1</sup>. Partial sequence analysis of the *tuf* gene showed that all these bacteria belong to *Lactobacillus parabuchneri* which is closely related to *Lactobacillus buchneri*, a bacterium associated with spoilage in plant-based fermented food and beverages but which is also used in silage fermentation.

**Objectives**

The aims were to develop a real-time PCR for the specific and quantitative detection of *L. parabuchneri* in milk and cheese as well as a genotyping methods for strain discrimination and biodiversity estimation.

**Methods**

We sequenced the genome of *L. parabuchneri* FAM21731 and twelve other strains.

The draft genome sequence was compared to publicly available genome sequences of *L. buchneri* and other related species to identify a gene sequence which seems to be unique for *L. parabuchneri*. Sequences were submitted to Tandem repeats finder and CRISPRFinder to assess hypervariable sequences and their potential for strain discrimination.

**Conclusions**

Results show that the real-time PCR system is specific for the detection and quantification of *L. parabuchneri* in dairy products and that the multiplex PCR based on hypervariable sequences can discriminate strains.

The use of these methods is of interest since it can be employed to locate potential sources of *L. parabuchneri* contamination on farms and in dairy plants.

**FEMS-2945**

**Food microbiology**

**DISCRIMINATION OF VIABILITY STATUS OF *SALMONELLA* TYPHIMURIUM CELLS INDUCED BY DISTINCT INACTIVATION METHODS USING A COMBINED FLOW CYTOMETRY, MALDI-TOF-MS AND MDA APPROACH**

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**Background**

Food chain contamination with *Salmonella* Typhimurium is one of the leading causes of foodborne illness worldwide. Rapid and reliable methods for the detection of foodborne pathogens and also discrimination profiles concerning its cells viability status are deeply required and of high importance to assure foodborne pathogens control in the food chain.

**Objectives**

The goal of this study is to ascertain the *Salmonella* Typhimurium viability status (viable, viable non-culturable and non-viable) induced by distinct inactivation methods through a combined Flow Cytometry, MALDI-TOF-MS and multivariate data analysis (MDA) approach.

**Methods**

Different inactivation treatments, such as batch pasteurization (65°C; 30 minutes), sodium hypochlorite (5% or 0.006% commercial bleach), commercial available disinfectant solution (AMUKINA), ethanol (100%) and methanol (100%) were tested in *Salmonella* Typhimurium LT2. Cell viability assessment was evaluated by CFU's counts and Flow Cytometry (Accuri C6) with LIVE(TO)/DEAD(PI) dyes. In addition, footprint patterns of different viability states were obtained by MALDI-TOF-MS using HCCA as a matrix. Data were analyzed by MDA.

**Conclusions**

All the inactivation methods tested resulted in a total death of *Salmonella* Typhimurium LT2 population, with the exception of bleach (0.006%) and pasteurization, the latter used in industrial facilities. These treatments induced cells viable non-culturable state, which is of concern, since under favorable conditions *Salmonella* Typhimurium could restore viability and eventually its pathogenicity potential. Moreover, we obtained discriminatory peptide/proteins profiles associated with different viability states of *Salmonella* Typhimurium, which combined with MDA could support the use of this approach to detect microbiological contaminants applicable for quality control purposes.



**FEMS-3129**

**Food microbiology**

## **INFLUENCE OF RAW MILK'S THERMODURIC DETERIORATING MICROBIOTA IN THE PASTEURIZED MILK'S SHELF LIFE**

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### **Background**

The refrigerated storage of raw milk at the mill has advantages such as cost savings with the sample collection and reducing the loss of raw material. However, inadequate procedures during production and milk collection are considered the main sources of contamination by spoilage and pathogenic microorganisms, resulting in economic and public health problems.

### **Objectives**

The work aims to enumerate the thermotolerant microbiota found in raw milk and in the milk after pasteurization, correlating it with the shelf life of the product.

### **Methods**

Nine raw milk samples were collected directly from the extension tanks, nearby Castro – PR. After LTLT pasteurization, a portion of raw milk and pasteurized milk were diluted in peptone saline solution in the concentrations: integral, 10-1, 10-2 and 10-3, sown in depth amid PCA inverted and incubated at 35°C for 48 hours. Petrifilm plates ECTM for the enumeration of total coliforms and E. coli were also used. An aliquot was plated in depth for thermoduric counting in the same as described for the days when milk was pasteurized. The milk used for plating thermoduric was also inoculated in Petrifilm ECTM for controlling the progression of coliforms and E. coli.

### **Conclusions**

Four samples showed a reduction in the count of thermoduric microbiota between pasteurization and 25 days of experiment. Two samples obtained less than one log cycle of microbial growth, while three samples showed growth of thermoduric microorganisms, more than one log cycle in the same sampling period.

**FEMS-3133**

**Food microbiology**

## **FENOLIC ACIDS EFFECTS ON PROBIOTIC BACTERIA GROWTH AND SURVIVAL**

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### **Background**

The consumption of probiotics for colonization and maintenance of the intestinal microbiota, regulation of immune mechanisms and decrease of lactose intolerance is strongly credited through data already obtained by investigation. It is also a widespread antioxidative activity of phenolic acids the prevention of diseases related to oxidative stress such as cancer and cardiovascular diseases.

### **Objectives**

Analyse de groth behavior and tolerance of *Lactobacillus plantarum* and *Lactobacillus casei* when exposed to diferent fenolic acids-

### **Methods**

We examined the behavior of two probiotic bacteria *Lactobacillus plantarum* and *Lactobacillus casei* when exposed to four different concentrations of six different phenolic acids: caffeic, ferulic, p-coumaric, hydroxybenzoic, vanillic and protocatechuich. Over 48h, analyzes were conducted by using a spectrophotometer.

### **Conclusions**

In presence of acids hydroxybenzoic and protocatechuich, the absorbance increased slightly in the deceleration phase at a concentration of 50 mg/L and 100 mg/L, and decreases to a concentration of 200 mg/L compared to the values absorbance of the control. While for *L. casei*, was observed for all acids tested, the absorbance values at a concentration greater than 50 mg/L, and values approximately equal to a concentration of 200 mg/L, compared to the control absorbance values. For protocatechuich acid, ferulic acid and p-coumaric acid has been possible to verify that the absorbance values were higher than controls at a concentration of 100 mg/L . In all cases, it was found that 200 mg/L showed an effect similar to the control except hydroxybenzoic acid.



**FEMS-1849**

**Food microbiology**

## **LISTERIA MONOCYTOGENES NMLR: ITS TRANSCRIPTOME AND ROLE IN STRESS RESPONSE**

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### **Background**

NmlR, a transcription regulator in MerR family, is involved in oxidative and nitrosative stress responses in *Neisseria gonorrhoeae* and *Haemophilus influenzae*. A homolog is also found in a foodborne pathogen *Listeria monocytogenes* (Im).

### **Objectives**

In this study, in order to study the role of NmlR<sub>Im</sub> in *L. monocytogenes*, a null *nmlR<sub>Im</sub>* strain was constructed. Transcriptomes of 10403S wild type (WT) and its isogenic null *nmlR<sub>Im</sub>* mutant (MT) strains grown to stationary phase were determined. Phenotypes of these strains in response to different stress conditions were also assessed.

### **Methods**

RNAseq experiments were performed on Ion Torrent platform and the transcriptomes of WT and MT during stationary phase were identified. WT and MT strains were exposed to acid stress and oxidative stress conditions. Role of NmlR<sub>Im</sub> in virulence-associated phenotype was also evaluated in intracellular growth assay using U937 human macrophage-like cell line. Cell sizes of WT and MT were measured using transmission electron microscopy.

### **Conclusions**

RNAseq result reveals that NmlR<sub>Im</sub> negatively regulates 46 genes and positively regulates 28 genes. NmlR<sub>Im</sub>-dependent genes are grouped into 28 operons, eight of which overlap HrcA regulon, another negative regulator in *L. monocytogenes*. Phenotypic characterization revealed that MT strain survived significantly less than WT under acid stress (pH 2.5) and oxidative stress (3% hydrogen peroxide). Null mutation in *nmlR<sub>Im</sub>* also resulted in significant decrease in cell length and impaired intracellular growth in U937. Our findings indicate that NmlR<sub>Im</sub> is not only involved in oxidative stress response but also contributes to acid persistence and intracellular growth via either direct regulation or co-regulation with other regulators.

**FEMS-0521**

**Food microbiology**

## **ADAPTATION AND TOLERANCE OF LACTOBACILLUS KEFIRANOFACIENS M1 TO VARIOUS ENVIRONMENTAL STRESSES**

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### **Background**

*Lactobacillus kefiranofaciens* M1, a novel probiotic strain isolated from Taiwanese kefir grains, has been demonstrated to possess anti-allergic, anti-asthmatic, anti-colitis and immunomodulatory effects in our previous in vitro and in vivo studies. These findings support that this strain has the potential to be applied in probiotic products. *L. kefiranofaciens* M1 must survive after processing, production and gastric passage to exert beneficial effects. Thus, the stress tolerance of *L. kefiranofaciens* M1 is important for its survivability.

### **Objectives**

This study was investigated the adaptation and tolerance of *L. kefiranofaciens* M1 to various environmental stresses.

### **Methods**

*L. kefiranofaciens* M1 was adapted to sublethal heat (37°C), cold (25°C), acid (pH 5), bile salts (0.05%), salts (0.1 M), ethanol (4%) and hydrogen peroxide (100 ppm) for 1 h and then challenged with lethal heat (52°C), cold (-20°C), acid (pH 3), bile salts (0.2%), salts (3 M), ethanol (20%) and hydrogen peroxide (1000 ppm), respectively.

### **Conclusions**

The results showed that adaptation to ethanol, hydrogen peroxide, salts and bile salts increased the tolerance of *L. kefiranofaciens* M1 to heat, cold, bile salts and ethanol stresses. Heat adaptation enhanced the tolerance to heat, bile salts and hydrogen peroxide. Acid adaptation caused an increased resistance to cold, acid, bile salts and hydrogen peroxide. Cold adaptation also induced cross-protection against cold, bile salts, salts and ethanol. However, ethanol- and cold-adapted cells decreased their survival after exposure to hydrogen peroxide. The occurrence of stress tolerance response in *L. kefiranofaciens* M1 varied depending on the type and condition of stress.

**FEMS-2059**  
**Food microbiology**

## **TRADITIONAL SOUR MILK FERMENTATION USING A POTENTIAL PROBIOTIC LACTOBACILLUS PLANTARUM STRAIN**

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### **Background**

Probiotic foods receive market interest as health-promoting, functional foods.

### **Objectives**

To evaluate the performance of a *Lactobacillus plantarum* strain with probiotic potential as co-starter culture in sour milk fermentation and its ability to give a final product with desirable sensory characteristics.

### **Methods**

UHT milk was inoculated with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* without (control case) or with *Lb. plantarum* and incubated in appropriate conditions (42°C, 6h). Fermented samples were stored at 4 and 12°C until the end of shelf life of product. Microbiological analysis was performed in parallel with pH and titratable acidity measurements as well as with sensory analysis of the product. The presence of the potential probiotic strain was determined using PFGE.

### **Conclusions**

On first day of storage, the population levels of all microbial groups exceeded 8 log cfu/ml for control and probiotic samples. During storage, the population levels were slightly elevated on probiotic samples compared to the control ones, whereas they were found to be above 8 log cfu/ml for all cases until the end of shelf life. The physicochemical properties (pH, titratable acidity) and the sensory characteristics of the potential probiotic product were similar to those of the control. According to PFGE the *Lb. plantarum* strain was recovered in high percentages during the products shelf life at both temperatures.

**Acknowledgment:** This work has been co-financed by the European Regional Development Fund (ERDF) of the EU and by National Resources under the Operational Program Competitiveness and Entrepreneurship (EPAN II), Action "COOPERATION 2011", Project "ProbioDairyMeat".

**FEMS-2069**

**Food microbiology**

**GREEK FUNCTIONAL YOGHURT: ENHANCING THE PRODUCTION PROCESS USING A SELECTED LACTOBACILLUS STRAIN WITH PROBIOTIC POTENTIAL**

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**Background**

Probiotic food products are in general fermented foods, with many studies reporting that the best matrices to deliver probiotics are dairy products, such as fermented milks and yogurt.

**Objectives**

To evaluate the performance of a *Lactobacillus plantarum* strain of dairy origin with probiotic potential as co-starter culture in Greek set-type yoghurt fermentation.

**Methods**

Low-pasteurized milk was inoculated with *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (control) or with the former starters plus *Lb. plantarum* (probiotic) and incubated in 42°C for 5h. Subsequently, samples were stored at 4 and 12°C until the end of shelf life of the product. Microbiological analysis was performed in parallel with physicochemical and sensory analysis of the product. The presence of *Lb. plantarum* was determined using Pulsed Field Gel Electrophoresis (PFGE).

**Conclusions**

The initial levels of all microbial groups were above 7 log cfu/g for control and probiotic samples. During storage, the population levels of all counts were slightly elevated on probiotic samples compared to the control ones, whereas they exceeded the 7 log cfu/g at all cases. Probiotic yoghurt produced with *Lb. plantarum* strain exhibited a better sensory profile, with a rich traditional taste, but with similar physicochemical properties to the control. According to PFGE, the *Lb. plantarum* strain was recovered in high percentages during the products shelf life at both temperatures.

**Acknowledgment:** This work has been co-financed by the European Regional Development Fund (ERDF) of the EU and by National Resources under the Operational Program Competitiveness and Entrepreneurship (EPAN II), Action "COOPERATION 2011", Project "ProbioDairyMeat".

**FEMS-2624**

**Food microbiology**

**IN VITRO ANTIBACTERIAL ACTIVITY AND COMPETITIVE INHIBITION EFFECT ON CACO-2 CELLS OF PROBIOTIC LACTOBACILLI ISOLATED FROM SARDINIAN DAIRY PRODUCTS**

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**Background**

The research of novel formulations with newly selected probiotic strains is important to satisfy the increasing request of the market and to obtain functional products with health-conferring properties. The most studied probiotic bacterial strains belong to the genus *Lactobacillus*, an important group of Lactic Acid Bacteria with functional and technological properties. Antagonistic activity toward potentially pathogenic microorganisms and adhesion to gut tissue are some of the main requirements when selecting successful probiotic strains.

**Objectives**

The aim of this work was to evaluate the antibacterial and anti-adhesion activity of *Lactobacillus plantarum* strains isolated from ewe's raw milk and their ability to *in vitro* colonize the intestinal epithelium. These strains were previously selected as potential probiotics because of their ability to survive at low pH values and physiological bile concentrations.

**Methods**

For detection of antagonistic activity, agar spot test and a well diffusion assay were performed toward *Escherichia coli* O157:H7, *Salmonella* Typhimurium and *Listeria monocytogenes*. The *in vitro* anti-adhesion effect was measured by a competitive inhibition assay on Caco-2 cells.

**Conclusions**

The results showed our strains having an excellent antibacterial activity against foodborne bacterial pathogens and anti-adhesion effect toward *E. coli* O157:H7 and *L. monocytogenes*, under the condition tested. Therefore, they showed interesting

probiotic characteristics. An *in vivo* study would be useful for a deeper evaluation of their properties, and their potential use in the development of novel functional food. This work has been supported by Regione Autonoma Sardegna (RAS, L.R. 7/2007).

**FEMS-0783**

**Food microbiology**

**COOPERATION BETWEEN SPECIES IN THE YOGURT CONSORTIUM IS  
AFFECTED BY THE MODULATION OF INTRACELLULAR PH THAT IS DRIVEN  
BY STREPTOCOCCUS THERMOPHILUS UREASE ACTIVITY**

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**Background**

The proto-cooperation between *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* in the yogurt consortium enhances the growth rate and size of each population. In contrast, the independent growth of the two species in milk leads to a slower growth rate and a smaller population size.

**Objectives**

In this study, we evaluated how urease activity of *S. thermophilus* might affect the intracellular pH of *L. delbrueckii*, modulating its bioenergetics during milk fermentation.

**Methods**

Urease-mediated intracellular alkalization of *S. thermophilus* and *L. delbrueckii* was evaluated by flow cytometry using cFSE as pH-dependent fluorescent probe. Lactose consumption and lactic acid production was followed *in vivo* by <sup>13</sup>C-NMR analysis. D-L lactic acid production in milk was measured enzymatically. For the evaluation of the pH-dependent glycolysis efficiency, glucose and lactic acid were measured by HPLC.

**Conclusions**

We observed that intracellular alkalization caused by urea hydrolysis or the addition of ammonia to milk boosted lactic acid production in *S. thermophilus* and in *L. delbrueckii* when the species were grown separately or in combination. Therefore, we propose that urease activity acts as an altruistic cooperative trait, which is costly for urease-positive individuals but provides a local benefit because other individuals can take advantage of urease-modulated pH. Compared to the interactions that are known to occur between *S. thermophilus* and *L. delbrueckii* in the yogurt consortium, the modulation of the bioenergetic efficiency due to the intracellular pH alkalization represents a new type of cooperation that directly affects the kinetic parameters of enzymes involved in homolactic fermentation in both the species.





**FEMS-1488**

**Food microbiology**

## **ESCHERICHIA COLI STRAIN DIVERSITY: SELECTING ISOLATES FOR USE AS PATHOGEN SURROGATES**

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### **Background**

*Escherichia coli* (*E. coli*) is commonly used as a surrogate for pathogens in research to identify sources of agricultural contamination and to characterize how pathogens persist on plant surfaces. However, *E. coli* strains are highly diverse, exhibiting differences in physical, chemical and biological properties which contribute to fitness in secondary habitats.

### **Objectives**

An important step in selecting *E. coli* for use as pathogen surrogates is to identify strains with characteristics (including adhesion, motility and biofilm formation) that contribute to their occurrence and survival in a manner similar to that of pathogens.

### **Methods**

To this end, strain-level differences in genotype (adhesions (*iha*, *agn43*, *eaeA* and *fimH*, *kpsMTII*) and siderophores (*iroN<sub>E.coli</sub>*, *chuA*)) and phenotype (biofilm formation, curli expression, and growth rate) were evaluated for isolates from livestock (swine, poultry, dairy) and water sources. Selected *E. coli* isolates (n=18 of 1,300), a common *E. coli* control strain (ATCC 25922), and *Salmonella* (ATCC 13311) were used in soil studies to evaluate adhesion to plastic, sandy loam or clay soils.

### **Conclusions**

Average adhesion of environmental isolates to plastic was two-fold higher than that of the control strain. *Salmonella* and one of the environmental isolates had adhesion rates seven-fold higher than the control strain. That isolate had extremely high adhesion to clay ( $91.3 \pm 2.7\%$ ) and sandy loam ( $91.7 \pm 0.1\%$ ) soils, but had none of the genes commonly associated with adherence or biofilm formation. These results suggest that *E. coli* strain choice should be an important consideration in its use as a pathogen surrogate.

**FEMS-2641**

**Food microbiology**

## **BACTERIAL TRANSCRIPTIONAL ADAPTATION TO NICHES: MOLECULAR AND METABOLIC RESPONSES OF LACTOBACILLUS PLANTARUM C2 IN PLANT SUBSTRATES**

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### **Background**

Raw fruits and some vegetables possess intrinsic chemical and physical features that make them particularly hostile environments for bacteria. To cope with environmental conditions, microorganism may adopt sophisticated adaptation mechanisms. The diversity of plant environments and of bacterial enzyme activities makes the microbial adaptation to plant niches markedly heterogeneous. *Lactobacillus plantarum* is a highly heterogeneous and versatile lactic acid bacteria frequently found or used in vegetables and fruits fermentation.

### **Objectives**

The aim of the study was to determine biological relevance of differentially expressed genes in *L. plantarum* C2 during fermentation of plant substrates, aiming to provide new insights into transcriptional response and niche adaptation.

### **Methods**

*L. plantarum* C2 was grown and stored in carrot or pineapple juices to mimic the chemical composition of the respective raw matrices. MRS broth was used as the control medium for optimal growth. Whole-transcriptome analysis based on customized microarray profiles has been used to determine altered transcription patterns in *L. plantarum* C2. These were compared with substrate utilization data gathered from high throughput phenotypic microarrays.

### **Conclusions**

Plant substrates exerted a transcriptional pressure and induced specific molecular and metabolic responses in *L. plantarum* C2. RNA and phenotypic microarray analyses revealed altered transcription patterns of genes encoding functions involved in primary metabolism, membrane transport, cofactors and vitamins metabolism, translation regulation, nucleotide metabolism, and fatty acid biosynthesis. Findings contribute to the description of bacterial transcriptional adaptation to niches, and provide a more solid basis for selection the most suitable starters for fermentation of targeted matrices.

**FEMS-0907**

**Food microbiology**

**IN VITRO ASSESSMENT OF ANTIBACTERIAL ACTIVITY OF RESINS FROM SOME WOODY PLANT BUDS AND SYNERGISTIC EFFECT BETWEEN STANDARD PHENOLIC COMPOUNDS**

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**Background**

With emerging of new and reemerging of old infectious diseases, urgent need for new antimicrobial agents arises. Resins are complex mixtures of phenolic and isoprenoid compounds secreted by plants to provide protection against predators and pathogenic microorganisms.

**Objectives**

The present study was aimed at investigating the *in vitro* antimicrobial activity of resins from some woody plants on the selected bacterial strains. To elucidate origin of antimicrobial activity, potential synergistic effect between standard phenolic compounds was investigated, also.

**Methods**

The samples of resins were collected from different regions in Serbia and dissolved in methanol. Well diffusion and broth microdilution methods were implemented for assessing antimicrobial activity.

**Conclusions**

Gram-positive bacteria exhibited strong susceptibility to samples from *Populus* sp. while being resistant on samples from other origin. The most sensitive strain was *B. subtilis*, towards samples collected from *Populus nigra* and *Prunus avium* (MIC was 0,05 mg/ml). The strains of *S. aureus*, MRSA and *L. monocytogenes* showed similar sensitivity to poplar samples with MIC values mostly under 1 mg/ml. *L. monocytogenes* was also susceptible to cherry buds samples. Sample U19 from white poplar had significant antimicrobial activity on all tested strains, with lowest detected MIC values. Synergistic effect of gallic acid, quercetin and caffeic acid on *B. subtilis*; gallic, caffeic, p-coumaric acid and naringenin on *E. faecalis*; and gallic, caffeic acid and naringenin on *S. flexneri* was observed. In conclusion, antimicrobial activity of resins is probably outcome of action of phenolic compounds.

**FEMS-2108**

**Food microbiology**

## **A COMPARISON OF LISTERIA SPP. PREVALENCE BETWEEN THE RAW FRESHWATER FISH FROM LAKE AND RETAIL MARKET IN LATVIA**

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### **Background**

Listeriosis is a foodborne infection caused by *Listeria monocytogenes*, which occurs due to ingestion of pathogen containing foodstuffs. *Listeria* spp. and *L. monocytogenes* are widespread in environment, but raw and processed fish products were found to be frequently contaminated with *Listeria* spp. Therefore fish are suspected to be a possible source for *Listeria* spp. including *L. monocytogenes* transmission to final food products.

### **Objectives**

The objectives of this study were to determine and compare the prevalence of *Listeria* spp. in raw freshwater fish from retail market and fish captured from lake in Latvia.

### **Methods**

A total of 51 raw freshwater fish samples were collected from lake (n = 11) and retail market (n = 39) in Latvia. A total amount of 25 g of fish skin, gut and muscle tissues of each fish was used for testing. Testing was performed in accordance to the International Organization for Standardization method (ISO 11290-1: 1996).

### **Conclusions**

The fish samples from lake were found to be *Listeria* spp. negative. In contrast, 69% of retail fish were found to be contaminated with *Listeria* spp. There were no significant differences between the prevalence of *L. innocua* and *L. monocytogenes* ( $p > 0.05$ ), while the prevalence of *L. ivanovii* and *L. welshimeri* was significantly lower than the prevalence of *L. monocytogenes* and *L. innocua* ( $p < 0.05$ ). The results

indicate that fish contamination with *Listeria* spp. may occur during fish handling, and the high prevalence of *L. monocytogenes* in raw fish at retail level can pose a threat for public health.

**FEMS-0608**

**Food microbiology**

**GENOTIPIIC DIVERSITY AND ANTIMICROBIAL RESISTANCE OF SALMONELLA TYPHIMURIUM STRAINS ISOLATED HUMANS AND FOOD BETWEEN 1983-2013**

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**Background**

The foodborne disease caused by *Salmonella* Typhimurium is a major health problem worldwide.

**Objectives**

The aims of this study were to genotype *S. Typhimurium* isolated in Brazil and to verify its antimicrobial resistance profiles.

**Methods**

A total of 92 *S. Typhimurium* strains, isolated from humans (43) and food (49), between 1983 and 2013 in Brazil, were typed by PFGE, ERIC-PCR and MLVA. Additionally, their antimicrobial resistance was evaluated. **Results:** The 92 *S. Typhimurium* strains were grouped in two clusters, by PFGE, PFGE-A and PFGE-B (subdivided in PFGE-B1 and PFGE-B2); by MLVA in two clusters, MLVA-A and MLVA-B (subdivided in MLVA-B1 and MLVA-B2). By ERIC-PCR, in three clusters, ERIC-A, ERIC-B and ERIC-C. The strains isolated from humans before the mid-1990s were allocated in the PFGE-A, PFGE-B1, PFGE-B2, MLVA-A, MLVA-B1, MLVA-B2, ERIC-A and ERIC-B. The strains isolated from humans after mid-1990s were distributed in the PFGE-B1, MLVA-B1, MLVA-B2 and ERIC-A. The strains isolated from food were distributed in the PFGE-A, PFGE-B1, MLVA-A, MLVA-B1, MLVA-B2, ERIC-A, ERIC-B and ERIC-C. Twenty-three (25%) strains were multidrug resistance.

**Conclusions**

The results suggest that the studied strains isolated from humans before the mid-1990s were genetically more diverse, which might indicate that selection of a more adapted *S. Typhimurium* subtype occurred after *S. Enteritidis* became the most prevalent serovar in Brazil. Regarding, the food strains the results suggest the current circulation of more than one subtype. The occurrence of multi-drug resistant strains isolated from food is an alert for the possible risk for human consumption.

**FEMS-0656**

**Food microbiology**

## **MOLECULAR CHARACTERIZATION AND RESISTANCE PROFILE OF CAMPYLOBACTER COLI STRAINS ISOLATED FROM DIFFERENT SOURCES IN BRAZIL**

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### **Background**

*Campylobacter coli* is an important causative agent of human diarrheal diseases worldwide. However, in Brazil is not frequently studied.

### **Objectives**

This study aimed to genotype and to investigate the presence of virulence genes and the resistance profile in *C. coli* strains.

### **Methods**

A total of 58 *C. coli* strains isolated from humans(10), animals(14), the environment(18) and food(9), between 1995-2011 in Brazil, were genotyped by PFGE. The presence of 16 virulence genes was searched by PCR. The resistance profile was obtained by MIC for erythromycin, ciprofloxacin, tetracycline and doxacycline. All the strains presented the genes *flaA*, *cadF* and *sodB*. The *cdtB*, *flhA*, *dnaJ* and *pldA* genes were observed in 20, 15, 10 and 6 strains, respectively. The *ciaB*, *iamA*, *cdtA*, *cdtC*, *docA*, *virB11*, *wlaN*, *racR* and *crsA* genes were not detected. PFGE grouped the strains in two main clusters with more than 45.4% of similarity. PFGE-A and PFGE-B clusters comprised 42 and 16 strains, respectively. In both clusters, strains of clinical and non-clinical sources were grouped in subclusters with a similarity value of more than 80%. The resistance profile showed that 19 strains, mainly of non-clinical sources, were resistant to at least one antimicrobial agent.

### **Conclusions**

The PFGE results confirmed the heterogeneity of the *C. coli* strains studied. However, the high similarity (>80%) among some strains of different origins suggests a possible contamination between clinical and non-clinical sources in Brazil. Because of these, the existence of resistant strains becomes a concern. The presence of important virulence genes indicates the pathogenic potential of those strains.

**FEMS-2242**

**Food microbiology**

**ANTIBIOTIC RESISTANCE PROFILES AND PREVALENCE OF ENTEROTOXIN GENES OF STAPHYLOCOCCUS AUREUS FROM FOOD HANDLERS**

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**Background**

*Staphylococcus* spp. are common members of the normal human flora. However, some *Staphylococcus* strains are recognized as human pathogens, in particular due to the production of several virulence factors and enterotoxins particularly relevant in food toxoinfections.

**Objectives**

Since many of the food toxoinfections by *S. aureus* are typically associated to cross-contamination, detection of *Staphylococcus aureus* was performed on food handlers.

**Methods**

Hand swabs from 167 food handlers were analysed for the presence of *Staphylococcus aureus* using Baird Parker agar and coagulase test, and genotypically confirmed by RT-PCR. A total of 26 strains were analysed using RT-PCR for the presence of virulence and enterotoxin genes, namely, *sea*, *seb*, *sec*, *sed*, *seg*, *sei*, *tsst1* and *pvl*. The same strains were phenotypically characterized in terms of antibiotic resistance using the disk diffusion method and antimicrobial agents from 15 different categories.

**Conclusions**

More than 11% of the samples were positive for *S. aureus*. A low prevalence of antibiotic resistant strains was found, with more than 55% of the strains being sensitive to all of the antimicrobial agents tested. However a high prevalence of resistance to macrolides was found, with 42% of the strains showing resistance to erythromycin. At least one of the virulence or enterotoxin genes was detected in 54% of the strains and the *seg* gene was detected in 42% of the strains.



**FEMS-1051**

**Food microbiology**

## **POTENTIAL GENETIC TARGETS FOR CONTROLLING CAMPYLOBACTER JEJUNI**

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### **Background**

Essential genes have been suggested to provide useful information for the development of new specific anti-infective agents in *C. jejuni*, as the major cause of bacterial gastroenteritis in humans worldwide (Stahl & Stintzi, 2011). To determine genetic regions that are present or highly prevalent in all *C. jejuni* strains could be also of interest as potential genetic targets for this purpose.

### **Objectives**

The aim of this study was to identify by microarray comparative genomic hybridization (MCGH) potential genetic targets for controlling *C. jejuni*.

### **Methods**

The prevalence of 1652 genes among 120 geographically diverse isolates obtained from different sources (human, chicken cattle, water and wild birds) was determined by MCGH using the BpG@S CJv3.0.0 microarray. As MCGH does not confirm the absence or divergence of the tested genes, the results were compared with those obtained by both MCGH and genomic sequencing in previous studies (Stabler *et al.*, 2013; van Tonder *et al.*, 2014).

### **Conclusions**

MCGH analysis detected 1366 genes (83%) with prevalence 90 % in the *C. jejuni* population studied. These genes included 816 genes (60%) recently proposed as core (van Tonder *et al.*, 2014), and 229 genes (28 %) proposed as essential gene candidates (Stahl & Stintzi, 2011; Metris *et al.*, 2011). A final list of 22 genes, including core and essential genes, was highlighted. All these genes encode products required for cell growth, and are therefore potential targets to consider for antimicrobial intervention strategies in *C. jejuni*.

**FEMS-0846**

**Food microbiology**

**THE EFFECT OF OSMOTIC DEHYDRATION PROCESS IN SUGAR BEET MOLLASES ON MICROBIOLOGICAL PROFILE OF CHICKEN MEAT**

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**Background**

Osmotic dehydration is a water removal process, based on soaking food (fruit, vegetable, meat and fish) in a hypertonic solution. Recent research has shown that use of sugar beet molasses as a hypertonic solution improves osmotic dehydration process from technological, nutritional and microbiological aspect.

**Objectives**

Effect of osmotic dehydration process on microbiological profile of chicken meat was investigated in order to determine the usefulness of this technique as pre-treatment for further treatment of chicken meat.

**Methods**

Fresh breast chicken meat was cut into cubes of 1x1x1 cm and contaminated with *Escherichia coli*, *Listeria monocytogenes* and *Salmonella* spp in laboratory conditions prior the process of osmotic dehydration. The process was conducted during 5 hours at 44°C in sugar beet molasses as an osmotic medium. All microorganisms were tested according to ISO methodology: *Salmonella* spp EN ISO 6579:2002, *Listeria monocytogenes* EN ISO 11290-2:1998, *Escherichia coli* ISO 16649-2:2001, *Enterobacteriaceae* ISO 21528-2:2004 and total number of microorganisms EN ISO 4833-1:2013.

**Conclusions**

The results of the microbiological profile of fresh, contaminated and osmotically dehydrated chicken meat have shown that the numbers of all examined microorganisms have significantly statistically reduced after the process.

From presented results it can be concluded that process of osmotic dehydration in molasses significantly improves microbiological profile of treated chicken meat and it is suitable pretreatment for further chicken meat processing, providing microbiologically safe intermediate product.

**FEMS-1579**

**Food microbiology**

**USE OF COMPARATIVE GENOMICS AND THIN LAYER CHROMATOGRAPHY TO IDENTIFY SECONDARY METABOLITES RESPONSIBLE FOR ANTI-GRAM NEGATIVE ACTIVITY IN BACILLUS AMYLOLIQUEFACIENS SUBSP. PLANTARUM**

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**Background**

Strong consumer demand for safe and high-quality products has continued to drive food and pet food producers to seek novel solutions targeting Gram-negative bacterial contamination, such as *Escherichia coli* and *Salmonella* spp.

**Objectives**

Since *Bacillus* spp. are known for their ability to produce antimicrobial compounds, culture supernatants of *Bacillus amyloliquefaciens* strains were evaluated and characterized for their antimicrobial properties. Furthermore, genome sequences were to be generated to provide insights to the mode of action.

**Methods**

Six *B. amyloliquefaciens* strains were used for the experiments. Titration of activity was conducted by measuring the halos in an agar well diffusion assay, employing an extensive range of bacteria and fungi. The effect of pH, heat and enzymes on the activity of the supernatants was expressed as a percentage of residual activity compared to non-treated samples. Draft genomes were generated by 454 FLX Titanium for each of the *Bacillus* strains and mined for the presence of secondary metabolites, which was furthermore confirmed with TLC-bioautography profiles of methanolic extracts.

**Conclusions**

All supernatants exhibited activity against important food related bacteria and fungi, including Gram negatives. Generally, considerable activity was retained after heating and at alkaline pH but not under acidic conditions. Whereas different enzymes had a variable effect on the antimicrobial activity. genomic data indicated that the *Bacillus* strains harboured gene clusters encoding proteins involved in the

production of an array of secondary metabolites, including several non-ribosomal peptide synthetases, polyketides, and ribosomally synthesized peptides, which could be responsible for the *in vitro* antimicrobial properties observed.

**FEMS-0689**

**Food microbiology**

**BAYESIAN MODEL FOR RISK ASSESSMENT OF CONSUMPTION  
CAMPYLOBACTER CONTAMINATED POULTRY IN FINLAND**

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**Background**

According to the Register of Infectious Diseases, supplied by the National Public Health Institute (THL), in 2013 *Campylobacter sp.* was the leading cause of human bacterial infection in Finland with 4059 cases registered (incidence 76 cases/100000 inhabitants). However, the real number of cases is assumed to be considerably higher than the registered cases numbers. The prevalence of *Campylobacter sp.* in animal-derived foods is affected by its prevalence in the production farms and in the animals. The contamination of the final product is also dependent on procedures carried out in abattoirs and during the various stages of the food production chain. The handling of food contaminated with *Campylobacter sp.* in domestic or industrial kitchens may also lead to cross-contamination, either directly from raw meat to products that will not undergo further cooking or indirectly via work surfaces, hands or utensils.

**Objectives**

Estimating the prevalence and concentration of *Campylobacter* in fresh (chicken, turkey) meat (sampled at retail), as well as presenting a novel Bayesian model to assess the probability of infection and illness.

**Methods**

Bayesian modeling of prevalence and concentration using Openbugs software based on results from detection and quantification of campylobacters (chicken: 608 samples from 226 batches) and (turkey: 558 samples from 185 batches) using modified NMKL 119:2007 and ISO 10272-2:2006.

**Conclusions**

Risk of *Campylobacter* exposure from Finnish fresh poultry meat is mainly due to undercooking or cross-contamination especially during summertime. The model can be used to assess e.g. relative risks, with quantification of uncertainty based on the data.

**FEMS-0648**

**Food microbiology**

**EFFECT OF ELECTRON BEAM IRRADIATION IN MINAS FRESCAL CHEESE  
ARTIFICIALLY CONTAMINATED WITH ENTEROHAEMORRHAGIC  
ESCHERICHIA COLI (EHEC) O157:H7**

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**Background**

The enterohemorrhagic *Escherichia coli* (EHEC) O157:H7 has been known to cause food-borne outbreaks. Cheeses are among the main food products incriminated in these outbreaks. Characterized as a perishable food, the Minas frescal cheese (MFC) has potential risk for O157:H7 transmission. As a result, the development of new technologies for its conservation has been studied. The use of irradiation has proved to be one of the most convenient options.

**Objectives**

The objective of the present study was to evaluate the effect of electron beam irradiation (doses of 1.0, 1.5 and 2.0 kGy) in MFC artificially contaminated with O157:H7.

**Methods**

Samples were produced to microbiological analyzes, one non-contaminated and non-irradiated, one contaminated and non-irradiated and three contaminated and irradiated. And to thiobarbituric acid reactive substances concentration (TBARS) analysis, one non-irradiated and non-contaminated and three non-contaminated and irradiated.

**Conclusions**

Artificially contaminated and irradiated MFC samples presented a shelf life of ten storage days at 4°C. The dose of 1.5 kGy reduced 2 log the number of O157:H7 and the doses of 1.5 and 2.0 kGy eliminated O157:H7 from the artificially contaminated samples. The TBARS assay was conducted to evaluate if the effect of irradiation could influence in the stability of the lipid fraction present in the cheese. All irradiated cheeses showed TBARS values, over the 40 day storage period, lower than in the non-irradiated sample. Results presented here demonstrate electron beam irradiation of MFC artificially contaminated with O157:H7 is a viable solution and a promising alternative to ensure the safety and quality of this food product.

**FEMS-1067**

**Food microbiology**

**BEHAVIOR OF SHIGA TOXIN-PRODUCING ESCHERICHIA COLI (STEC) O157:H7 AND O113:H21 UNDER THE EFFECT OF DIFFERENT LEVELS OF PH AND TEMPERATURE**

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**Background**

Shiga toxin-producing *Escherichia coli* (STEC) cause hemorrhagic colitis, hemolytic uremic syndrome and bloody diarrhea.

**Objectives**

This work aimed to evaluate the effect of two individual factors (pH and temperature), and their interaction, on the multiplication and survival of STEC (O157:H7 and O113:H21) using statistics as a tool for development of the experimental design and the analysis of results.

**Methods**

Approximately 10<sup>8</sup> CFU per ml were inoculated into Trypticase Soy Broth at pH conditions (4.0, 4.5, 5.5, 6.5 and 7.0) and temperature (6, 10, 20, 30 and 35°C). The experimental design was prepared by a central composite design, generated by the software Statistica 7 (StatSoft, OK, USA), and given 11 trials including three central points and four axial points. Assays were performed in 22 h.

**Conclusions**

Were generated equations that model the bacterial behavior from a multiple linear regression model. In the study with the RJ 581 strain (O157:H7), variables, individually and combined, showed linear significant effect on the response, the effect of the variable pH being the most relevant, with R<sup>2</sup> equal to 0.94. Explaining the optimal conditions for the multiplication of the RJ 581 strain values are closer to pH 7.0 and 35°C. In the study with the RJ 702 strain (O113:H21), we observed that the independent and combined variables had no significant effect on the response. The R<sup>2</sup> of the model was 0.4, showing a low suitability of experimental design to RJ 702 strain.

**FEMS-3011**

**Food microbiology**

## **LACTIC ACID BACTERIA AS ANTAGONIST AGENTS TO FOODBORNE PATHOGENS ON FRESH-CUT 'GOLDEN DELICIOUS' APPLES**

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### **Background**

Food safety is a major concern in fresh-cut industry. Fresh-cut produce can become contaminated with foodborne pathogens during manipulation. Application of natural antimicrobial substances provides new opportunities for the control of pathogenic bacteria. Lactic acid bacteria (LAB) are known to have antagonistic effect against sensitive bacteria species/strains by the secretion of substances with antimicrobial activity.

### **Objectives**

The main objective of this work was to do a screening of 37 LAB isolates for antimicrobial activity on minimally processed 'Golden delicious' apples against the foodborne pathogens *Escherichia coli* O157:H7 NCTC 12900, *Salmonella enterica* subsp. *enterica* Michigan ATCC BAA-709, *Cronobacter sakazakii* ATCC 51329 and *Staphylococcus aureus* ATCC 25923.

### **Methods**

LAB studied were isolated from fresh-cut fruit purchased in the market. One g of fresh-cut apple was inoculated with the LAB followed by the inoculation of the pathogen at a concentration of  $10^8$  cfu/mL. Samples were incubated at 30 °C for 48 h and after the recovery of the pathogens were made on selective/differential mediums. Fresh-cut apples inoculated with only the pathogen were used as control.

### **Conclusions**

From the 37 LAB isolates 18 were capable to reduce all the pathogens more than 2 log cfu/g and 5 achieved a reduction of more than 3 log cfu/g for all the pathogens. *S. enterica* and *C. sakazakii* showed reduction with all the isolates studied and *E. coli* and *S. aureus* showed reduction with 36 of the 37 isolates. Results obtained support the potential use of LAB as biocontrol agents against foodborne pathogens in fresh-cut fruit.





**FEMS-2728**

**Food microbiology**

## **FERMENTATION PROPERTIES AND POTENTIAL PREBIOTIC ACTIVITY OF A HIGH PURITY GOS ON IN VITRO GUT MICROBIOTA PARAMETERS IN HEALTHY INDIVIDUALS**

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### **Background**

Most *in vitro* and *in vivo* studies involving prebiotic oligosaccharides have been carried out using inulin and its fructooligosaccharide (IFS) derivatives, as well as various galactooligosaccharides (GOS). It has been shown that these food ingredients have the ability to improve selectively the growth of bifidobacteria and, consequently, lead to important changes in the gut microbiota composition that may confer health benefits to the host. Bimuno®, (Clasado Ltd., Buckinghamshire, UK) is a 50% mixture containing galactooligosaccharides (B-GOS) having multiple biological health activities within the colonic environment, beyond the stimulation of bifidobacteria and lactobacilli at genus level and it has been tested in different *in vitro* and *in vivo* studies.

### **Objectives**

The present study aimed to determine the *in vitro* potential of a new high GOS content version of Bimuno (65% GOS content), comparing to B-GOS used as positive control, in a pH and volume controlled dose response batch culture experiments. Three different doses of the high purity GOS (2.75, 1.38 and 0.92 g) were tested.

### **Methods**

Changes in the gut microbiota during a time course were identified by fluorescence *in situ* hybridisation (FISH), whilst the small-molecular weight metabolomic profiles and short chain fatty acids (SCFAs) were determined by NMR analysis and Gas Chromatography (GC), respectively.

### **Conclusions**

The High purity GOS showed positive modulation of the microbiota composition after 4h fermentation at the lowest dose compared to B-GOS. Moreover, the administration of the specific GOS induced a significant increase in acetate as the major SCFAs synthesized compared to propionate and butyrate concentrations.

**FEMS-1640**

**Food microbiology**

**COMMON AND DIVERGENT FEATURES OF THE HEAT-RESISTANT SPOILAGE  
PROTEASE PRODUCING MICROBIOTA IN MILK FROM TWO DIFFERENT UHT-  
MILK PRODUCING COUNTRIES, BRAZIL AND BELGIUM**

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**Background**

The majority of the bacteria from raw milk are inactivated by heat treatments commonly used in dairy industry (pasteurization or ultra-high temperature (UHT)). However, these treatments do not destroy heat-resistant spoilage enzymes such as proteases produced by certain psychrotrophic bacteria before processing.

**Objectives**

The first objective was to identify the main proteolytic micro-organisms in raw milk from Belgium and Brazil, two countries with a large UHT-milk production but under different primary production conditions which can influence the microbiota composition of raw milk. Secondly, the diversity of the protease enzymes was investigated in order to characterize the target of future spoilage detection assays .

**Methods**

Raw milk samples were stored at simulated conditions of farm storage and transport according to Brazilian and Belgian regulations, respectively. The highly proteolytic strains were polyphasically identified. Their protease enzymes were characterized for heat resistance, proteolytic activity in milk, casein zymogram analysis with MALDI-TOF identification of protein bands, and for gene sequence diversity.

**Conclusions**

The *Pseudomonas fluorescens* group together with *Ps. fragi* and *Ps. lundensis* were dominant in Belgian raw milk, while in Brazil, almost the same *Pseudomonas* spp. together with *Serratia liquefaciens* were dominant, the latter being an indication of less hygienic conditions during milking. Belgian isolates showed overall a significant higher proteolytic activity than most Brazilian isolates. The proteolytic activity from both sources was resistant to UHT conditions with the one from *S. liquefaciens* being the most heat resistant. It seems that the spoilage protease from *S. liquefaciens* is

unrelated to the heterogeneous *Pseudomonas* AprX protease.

**FEMS-1661**

**Food microbiology**

**POLYPHASIC IDENTIFICATION OF THE DOMINANT MICROBIOTA OF PEELED BROWN SHRIMP (CRANGON CRANGON) STORED UNDER MODIFIED ATMOSPHERE PACKAGING AT 4 °C WITHOUT PRESERVATIVES**

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**Background**

Brown shrimp are, like many seafood products, prone to rapid spoilage. The main factor limiting shelf life is microbiological activity. Specifically for brown shrimp without preservatives, the microbiota is dominated by the genera *Pseudoalteromonas* and *Psychrobacter* when stored under iced aerobic conditions, but the spoilage bacteria of shrimp when cool stored under modified atmosphere (MAP) conditions without preservatives, a product which is gaining popularity among consumers, are still unknown.

**Objectives**

The main objective was to evaluate the variation throughout the year and during storage in the microbial community of MAP packaged cooked and machine peeled shrimp (*Crangon crangon*) without preservatives and the description of the dominant spoilage microbiota after 7 days of shelf life at 4 °C.

**Methods**

Three series of shelf life experiments resulting from three sampling periods of cooked shrimps throughout the year were conducted with mechanically peeled shrimp stored in a modified atmosphere (40% CO<sub>2</sub> and 60% N<sub>2</sub>). Culture-dependent and – independent methods, notably (GTG)<sub>5</sub> clustering followed by 16S rRNA gene sequencing of isolates and 16S rRNA gene based DGGE in combination with metagenomics, were used to monitor the bacterial population in brown shrimp without preservatives.

**Conclusions**

According to culture dependent identification the microbiota of brown shrimp was dominated by *Arthrobacter bergerei*, *Shewanella putrefaciens*, *Aliivibrio* spp., *Psychrobacter* spp., *Brochothrix thermosphacta* and *Vagococcus salmoninarum*. Using the culture independent approach the dominating genus after 7 days of storage was *Carnobacterium*. Most of the micro-organisms identified through the complementary approach are known to contribute to spoilage, but for some (e.g. *V.*

*salmoninarum*) the spoilage potential is yet unknown.

**FEMS-0758**

**Food microbiology**

## **DNAJ GENE AS A MOLECULAR DISCRIMINATOR WITHIN THE LACTOBACILLUS CASEI GROUP**

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### **Background**

The current taxonomy of the *Lactobacillus casei* group comprises three closely related species: *L. casei*, *L. paracasei* and *L. rhamnosus*. Some strains of these species have probiotic features and are now widely used in the food industries. However, neither phenotypic nor the most frequently applied genotypic marker (16S ribosomal DNA) provides sufficient resolution for accurate identification of *L. casei* group.

### **Objectives**

The aim of this study was to use the *dnaJ* gene as a molecular marker for species-level discrimination and identification within the *L. casei* group.

### **Methods**

The degenerate primers (LcasDnaj-F1/R1) were designed for *dnaJ* gene amplification and sequencing. The relationships between the members of *L. casei* group were conducted based on sequence similarity and phylogenetic analysis. The species-specific primers were designed by analysing the highly variable regions of the *dnaJ* gene.

### **Conclusions**

Within the *L. casei* group, the nucleotide sequence similarity of *dnaJ* gene was significantly lower than that of 16S rRNA gene, and all examined strains could be clearly distinguished. In addition, the species-specific primers were developed, which were then employed for two-plex minisequencing analysis, and were shown to be specific for *L. paracasei* and *L. rhamnosus*. Our data indicate that the phylogenetic relationships in the *L. casei* group can be resolved using *dnaJ* gene sequencing, and the species of *L. paracasei* and *L. rhamnosus* can be identified using novel species-specific minisequencing assay.





**FEMS-1747**

**Food microbiology**

**INFLUENCE OF PH ON GENE EXPRESSION IN LACTOBACILLUS  
PARABUCHNERI FAM21731**

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**Background**

The formation of biogenic amines in cheese can occur as a result of the presence of bacteria possessing amino acid decarboxylase activity. One of the most important biogenic amines in cheese is histamine, since it can cause serious human health problems upon ingestion.

**Objectives**

To better understand the factors contributing to histamine accumulation, we isolated histidine decarboxylase positive lactic acid bacteria from various raw-milk cheeses with elevated histamine levels. All isolates belonged to the species *Lactobacillus parabuchneri*. Various factors, such as starter culture, proteolysis during ripening, storage temperature, salt concentration and pH can affect histamine formation in cheese. For cheeses made from raw milk, a thermal treatment of the milk prior to cheese making to reduce the numbers of histamine producing *L. parabuchneri* is not an option. Other strategies could be developed to prevent accumulation of biogenic amines if the metabolism of these bacteria is known in more detail.

**Methods**

RNA from *L. parabuchneri* FAM21731 grown at various pH was isolated and sequenced on an Ion Torrent PGM sequencer.

**Conclusions**

Whole transcriptome analysis of *L. parabuchneri* was established using next generation sequencing technology. The methodology helps us to better understand how cheese making technology and choice of starter culture influence the metabolism of histamine producing *L. parabuchneri* strains.

**FEMS-1842**

**Food microbiology**

**EXPRESSION PROFILING OF *PEDIOCOCCUS ACIDILACTICI* FAM18098 WITH A FOCUS ON THREONINE AND SERINE CATABOLISM**

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**Background**

Proteolysis and amino acid catabolism are the most important events taking place during cheese ripening. Microorganisms play a crucial role in these biochemical changes. Among various lactic acid bacteria *Pediococcus acidilactici* is often found in cheese at the end of ripening. Little is known if this species contributes to protein break down and flavor development in cheese. When *Pediococcus acidilactici* FAM18098 was used as adjunct culture in cheese making, we observed that it degraded arginine, serine and threonine and synthesized ornithine, alanine and 2-aminobutyrate during cheese ripening.

**Objectives**

*P. acidilactici* FAM18098 did not degrade serine and threonine under laboratory conditions using MRS broth, a broth usually used for lactic acid bacteria in the laboratory. By changing the composition of media, we found a medium in which this strain showed an amino acid metabolism similar to the one observed in cheese. By analyzing the transcriptome of *P. acidilactici* FAM18098 we want to better understand the transcriptional regulation of genes involved in amino acid metabolism and to find experimental conditions that are similar to the ones of cheese.

**Methods**

RNA from *P. acidilactici* FAM18098 grown in various media was isolated and sequenced on an Ion Torrent PGM sequencer.

**Conclusions**

We established a protocol to analyze and compare whole transcriptomes of *P. acidilactici* with next generation sequencing technology. With this methodology we studied genes that are involved in amino acid metabolism under conditions that probably mimic a cheese environment.



**FEMS-1356**

**Food microbiology**

## **TRANSFER OF *L. MONOCYTOGENES* FROM DIFFERENT FOOD CONTACT SURFACES TO CHEESES**

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### **Background**

The food contact surfaces are subject to contamination by pathogens that could lead to cross-contamination by transfer events to others food products. However, the European regulation n° 1935/2004 of 27<sup>th</sup> October 2004 (Anonymous, 2004) specifies that materials intended for safe food contact must not interfere with foodstuff characteristics.

As a traditional and natural material, wooden boards are traditionally used as as a “technological tool” during cheeses ripening process. In France, wood is authorized for food contact by the French Arrêté November 1945 (Anonymous, 1945) and the information note of DGCCRF 2012-93(Anonymous, 2012).

### **Objectives**

The aim of this study was to determine the behavior of wooden surfaces in direct food contact, contaminated by a well-known risk along the production chain of dairy products: *Listeria monocytogenes*.

### **Methods**

For this purpose, a protocol was defined and new spruce boards were inoculated by *L. monocytogenes* solution at a concentration of 10<sup>5</sup> CFU/cm<sup>2</sup> and the microbial transfer to pressed non-cooked cheeses was studied. Factors such as cheese time contact, wood moisture content and age of the cheeses were tested.

A comparison of transfer quantification, with other materials surfaces: glass plates and plastic sheets with inclined meshes, used in cheese production, was also realized among the same conditions.

### **Conclusions**

The results showed for all tested surfaces a transfer yield below 3% (CFU/cm<sup>2</sup>) in the first hour. No differences were found for older cheeses or for higher wood moisture

content. In conclusion, wooden shelves are, as much as, safety for food contact than plastic or glass surfaces.

**FEMS-1049**

**Food microbiology**

## **BIOFILM FORMATION AND VIRULENCE GENOTYPES OF ARCOBACTER BUTZLERI ISOLATED FROM FOOD PRODUCTS**

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### **Background**

Manipulation or consumption of raw or poorly cooked foods of animal origin is an important source of human infection with the emerging food and waterborne pathogen *Arcobacter butzleri*. The ability of this bacterium to form biofilm favors the transmission and its adherence to food and food-contact surfaces possibly favors cross-contamination. Preventing cross-contamination is a key factor in Food Safety. Besides, studying the virulence properties of any potentially pathogenic foodborne bacteria is essential for the consumer safety.

### **Objectives**

The aim of this study was to determine the biofilm formation ability of 42 *A. butzleri* isolates obtained from different food products and to study the prevalence of ten putative virulence genes among adherent isolates.

### **Methods**

Biofilm formation was assayed by microtiter plate method (Teh *et al.*, 2010) and categorized according to Naves *et al.* (2008). Putative virulence genes were studied by PCR (Doudah *et al.*, 2012; Karadas *et al.*, 2013).

### **Conclusions**

Eight isolates were categorized as weakly adherent and one isolate as strongly, under the tested conditions. Adherence was not related with a specific food product. The genes *cadF*, *ciaB*, *cj1349*, *mviN*, *pldA* and *tlyA* were detected in all adherent isolates; whilst *iroE* (4/9), *hecA* (1/9) and *hecB* (2/9) were detected only in few. *irgA* was not detected.

The occurrence of adherent *A. butzleri* isolates possessing virulence markers in food products of animal origin is an important factor to consider for the foodborne illness risk assessment.



**FEMS-1050**

**Food microbiology**

**SOURCE TRACKING GENETIC MARKERS IN CAMPYLOBACTER JEJUNI BY MLST AND MCGH**

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**Background**

*Campylobacter jejuni* is a major cause of food-borne diarrheal illness in humans worldwide. Despite being a well known food and water-borne zoonosis, establishing the source of campylobacteriosis is still complicated (Hepworth *et al.*, 2011).

**Objectives**

The aim of this study was to find genetic markers for clonal complex (CC) and source attribution in *C. jejuni*.

**Methods**

Multilocus sequence typing (MLST) and microarray-based genomic hybridization (MCGH) were applied to 69 *C. jejuni* isolates from different sources (wild birds, surface and wastewater, and human and bovine feces). Oligonucleotide DNA microarrays (BµG@S CJv3.0.0) were used to compare *C. jejuni* isolates, with *C. jejuni* NCTC 11168 and RM1221 as reference strains.

**Conclusions**

The population was genetically diverse. MLST differentiated the isolates into 38 sequence types (STs), of which 29 were included in seven different CCs. Ten new alleles were identified, which resulted in the assignment of 12 new STs.

MCGH confirmed most of the previously described variable and/or plasticity regions (Parker *et al.*, 2006; Hepworth *et al.*, 2011; Stabler *et al.*, 2013). Combining MLST and MCGH results allowed us to detect several potential genetic markers associated with both, specific CCs and certain sources of isolation. More accurately, the genes *cas2*, *cas1*, *Cj0659c-Cj0660c*, *Cj0887c*, *Cj1041-Cj1042*, *hsdM* and *hsdR* related with the CCs ST-21, ST-42, ST-177 and ST-677; and the genes *glpT*, *ldh* and *Cj1562* related with isolates from wild birds.



**FEMS-1054**

**Food microbiology**

## **GENETIC DIVERSITY AND HORIZONTAL GENE TRANSFER IN FOODBORNE ARCOBACTER CRYAEROPHILUS ISOLATES**

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### **Background**

*Arcobacter* species have been recognized as potential food- and waterborne pathogens, and are implicated in human enteritis. The wide genetic diversity reported among *Arcobacter* species reflects the continuous evolving nature of these pathogens.

### **Objectives**

The aim of this work was to determine the genetic diversity and virulence gene content of 10 *Arcobacter cryaerophilus* isolates obtained from clams, mussels and raw cow milk obtained in Vitoria, Spain.

### **Methods**

Genetic diversity was studied by multilocus sequence typing (MLST). The obtained genotypes were analyzed using the MEGA 5.1 software, and possible recombination events were evaluated in silico using the RDP3 software. Recombination events were considered when detected by at least 3 methods. The presence of nine putative virulence genes was determined by PCR.

### **Conclusions**

A total of 9 sequence types (ST) not previously described were identified among the 10 isolates. All the isolates harbored virulence genes. The most frequent were *ciaB* and *mviN* (100%), followed by *tlyA* (40%) and *hecA* (30%). Due to the incongruent phylogenetic relationships observed for *glnA* and *glyA* genes, recombination analysis was performed. This analysis detected potential recombination events in the ST413, ST415 and ST416 isolates, probably derived from interspecies recombination between *A. cryaerophilus* and *A. skirrowii*.

From these results, we conclude that MLST typing showed high strain diversity and that recombination should be considered as a potentially relevant mechanism generating genetic diversity in *A. cryaerophilus*.

**FEMS-1758**

**Food microbiology**

## **FURTHER EVIDENCE FOR STAPHYLOCOCCAL FOOD POISONING OUTBREAKS CAUSED BY SEG AND SEI**

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### **Background**

Staphylococcal food poisoning represents the most prevalent foodborne intoxication worldwide. It is caused by oral intake of enterotoxins preformed by *Staphylococcus aureus* in food. The relevance of enterotoxins SEG and SEI in outbreaks of staphylococcal food poisoning is controversially discussed. Although SEG and SEI elicit emesis in a monkey feeding assay, there has been no conclusive proof of their emetic activity in humans.

### **Objectives**

In this study, we present novel evidence suggesting that SEG and SEI can cause staphylococcal food poisoning and describe the special challenges associated with investigating outbreaks linked to SEG and SEI.

### **Methods**

We analyzed all outbreaks registered with the Swiss Federal Office of Public Health, in which only *Staphylococcus aureus* strains harboring *seg* and *sei* linked to typical signs of staphylococcal food poisoning were isolated (n = 3).

### **Conclusions**

The outbreaks were caused by consumption of raw goat cheese, potato salad, and semi-hard goat cheese, and were linked to strains assigned to CC45 (*agr* type I), CC30 (*agr* type III), and CC9 (*agr* type II), respectively. Investigation of the outbreaks was particularly challenging as, in contrast to classical staphylococcal enterotoxins, there are currently no suitable methods for detection of SEG and SEI in food and feces. Our data provides strong evidence suggesting that SEG and SEI can cause staphylococcal food poisoning in humans. Further research will be necessary to determine the individual role of SEG and SEI in outbreaks.

**FEMS-1796**

**Food microbiology**

**FOOD POISONING OUTBREAK AMONG CHILDREN AND STAFF AT SWISS BOARDING SCHOOL DUE TO RAW MILK CHEESE CONTAMINATED WITH STAPHYLOCOCCUS AUREUS OF GENOTYPE B**

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**Background**

In 2014, an outbreak of staphylococcal food poisoning occurred at a Swiss boarding school due to consumption of Tomme, a soft cheese produced from raw cow milk. All 14 persons that had ingested the cheese fell ill, among them ten children.

**Objectives**

We present a food poisoning outbreak caused by raw milk cheese contaminated with a *Staphylococcus* (*S.*) *aureus* strain of genotype B.

**Methods**

The Tomme cheese was tested for presence of staphylococcal enterotoxins and *S. aureus* strains isolated from the cheese were characterized by *spa* typing and a DNA microarray.

**Conclusions**

The cheese exhibited low levels of staphylococcal enterotoxin A (> 6 ng SEA/g cheese) and high levels of staphylococcal enterotoxin D (> 200 ng SED/g cheese). A total of 10<sup>7</sup> CfU coagulase-positive *Staphylococci* per gram cheese were detected, with three different *S. aureus* strains being present at levels higher than 10<sup>6</sup> CfU/g. A strain exhibiting *sea* and *sed* was identified as the source of the outbreak. The strain was assigned to *spa* type t711 and CC8 and exhibited genetic criteria consistent with the characteristics of a genotype B strain. This genotype comprises bovine *S. aureus* exclusively associated with very high within-herd prevalence of mastitis and has been described as a major contaminant in Swiss raw milk cheese. It is highly likely that the raw milk used for production of the Tomme was strongly contaminated with *S. aureus* and that levels further increased due to growth of the organism and physical concentration effects during the cheese making process.

**FEMS-1801**

**Food microbiology**

**TEMPORAL EXPRESSION OF THE STAPHYLOCOCCAL ENTEROTOXIN D GENE UNDER NaCl STRESS CONDITIONS ENCOUNTERED DURING FOOD PRODUCTION AND PRESERVATION**

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**Background**

*Staphylococcus aureus* represents one of most osmotolerant food-borne pathogens. While its growth is repressed by competing bacteria, the organism exhibits a growth advantage at increased salt concentrations. Staphylococcal enterotoxin D (SED) leads to vomiting and diarrhea upon consumption. To date, the effect of NaCl on both *sed* expression and its regulatory control are unclear.

**Objectives**

We aimed to determine the impact of NaCl stress on *sed* expression and the influence of *agr*, *sarA*, and *sigB* on *sed* expression under NaCl stress.

**Methods**

Using qPCR, temporal expression of *sed* in LB and LB with 4.5% NaCl was compared, as well as *sed* expression between wild type strains and isogenic  $\Delta agr$ ,  $\Delta sarA$ , and  $\Delta sigB$  mutants.

**Conclusions**

In general, NaCl stress led to decreased *sed* expression. However, one strain exhibited a trend towards increased *sed* expression under NaCl stress. One  $\Delta sarA$  mutant each showed decreased *sed* expression in the early stationary and increased *sed* expression in the stationary growth phase under NaCl stress. No significant effect of *agr* on *sed* expression was detected, and only one  $\Delta sigB$  mutant showed a significant decrease in *sed* expression in the early stationary phase under NaCl stress. These findings suggest high strain-specific variation in *sed* expression and its regulation under NaCl stress.

**FEMS-3061**

**Food microbiology**

**TRACKING CHANGES OF BACTERIAL COMMUNITY COMPOSITION IN A  
LARGE INTESTINAL MODEL SYSTEM BY QPCR**

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**Background**

In vitro digestion models are simplified artificial systems to simulate digestion processes in humans/animals. Our digestion system aimed to study structural changes, digestibility and bioavailability of food components and behaviour of microbes. The model consisted of four stages, imitating the environment of mouth, stomach, small intestine and large intestine.

**Objectives**

Selective agar plates ensured reliable enumeration of bacterial colonies, but cultivation took 2-3 days, especially in case of the slowly growing species (e.g. *Bifidobacterium*), therefore, qPCR technique was applied for enumeration of bacteria.

**Methods**

Microbial activity was investigated in the large intestinal phase. Bacterial community consisted of two probiotic (*Bifidobacterium* and *Lactobacillus*) and four neutral/potential pathogenic species (*Clostridium*, *Bacteroides*, *Enterococcus*, *Escherichia*), found frequently in the human large intestine. The effect of food components (including prebiotic carbohydrates) on proliferation and survival of bacteria was evaluated on selective agar plates and by qPCR, using newly designed species-specific primers in the latter case.

**Conclusions**

The use of species specific primers for qPCR-based quantitation was successful, the number of each bacteria was determined precisely. Differences between the plate count and qPCR techniques were within an order of magnitude range in most cases. However, selective agar plates are still widely used for enumeration of bacteria in laboratories, other methods like qPCR are used more and more frequently, due to their speed and reliability. Our experiments showed that carefully selected primers were able to ensure the specificity for quantitation of bacteria from mixed cultures, in the presence of interfering compounds, such as digestion juice.

**FEMS-2713**

**Food microbiology**

**EFFECT OF OZONATED AND CHLORINATED WATER ON MICROBIOLOGICAL LOAD AND SENSORY PROPERTIES OF FRESH LETTUCE (*LACTUCA SATIVA*) AND BROCCOLI (*BRASSICA*)**

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**Background**

Ozonated and chlorinated water used as potential antimicrobials agents in food industry. Since 2001, when US Food and Drug Administration (FDA) announced the permission of Ozon's usage as a suitable antimicrobial agent for food, many investigations have been applied (Singh et al. 2002, Trinetta et al. 2011).

**Objectives**

The objectives of this experimental work are to determine the effectiveness of ozonated (33.3 mg/l) and chlorinated (50 mg/l) water on the reduction of microbiological load on two vegetable species. The project's target is to estimate the load of four pathogen microorganisms (*Echerichia coli*, *Listeria monocytogenes*, *Salmonella* spp, *Enterobacteriaceae*) at the different market points and highlight if those specific vegetables are safe for the consumers. One more target is to remark any changes at the qualitative and sensory characteristics. Furthermore, was examined the microbiological load of vegetables when they have been washed with tap, chlorinated and ozonated water.

**Methods**

The methods which used for the bacteria are Health Protection Agency (HPA), ISO 11290-1:1996/Amd 1:2004, ELOT EN ISO 6579:2003/TC1:2004, Statutory Instrument SI 2383, 1989 and BS 4285:3.7. The part of the sensory analysis consists of a specific panel with ten people, who tasted and evaluated vegetables, after treated and washed with three treatments.

**Conclusions**

According to the results was proved that the region of origin probably is important for the surcharge of the microbiological load, but this is not affected by the sampling point. Ozonated water has the best performance for all samples for the microbiological control and sensory analysis. In general, broccoli have less Enterobacteriaceae load than lettuce.



**FEMS-0945**

**Food microbiology**

**DETERMINATION OF KILLER ACTIVITIES OF THE YEAST STRAINS ISOLATED FROM NATURAL FERMENTATION MEDIA OF GEMLIK CULTIVAR BLACK OLIVES**

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**Background**

Yeasts are common microorganisms present in spontaneous table olive fermentation. Killer yeasts secrete protein toxins that are lethal to sensitive strains. *Wickerhamomyces anomalus*, *Kluyveromyces marxianus*, *Pichia guilliermondii*, and *Saccharomyces cerevisiae* strains isolated from olive brines have been shown to produce killer toxins with a broad spectrum of activity against spoilage microorganisms of table olives. The killer characteristics of the yeasts have been applied to combat undesirable yeasts during the production of table olive.

**Objectives**

The aim of this study was to determine killer activities of the yeast strains isolated during natural fermentation process of 'Gemlik' cultivar olives grown in Akhisar and Iznik regions of Turkey. The effect of regional differences on killer yeast profile of Gemlik olives was also investigated.

**Methods**

A total of 54 endogenic yeast strains were investigated for killer activity. These were belonging to 7 genera as; *Candida*, *Aureobasidium*, *Debaryomyces*, *Cryptococcus*, *Kloeckera*, *Pichia* and *Clavispora*. Killer activity was detected by agar diffusion assay. In the method, two killers; *S.cerevisiae* NCYC 232 (K<sub>1</sub>), *S.cerevisiae* NCYC 738 (K<sub>2</sub>) and a sensitive strain; *S.cerevisiae* NCYC 1006 were used as reference yeasts.

**Conclusions**

From 54 strains, 40 were found as killer and 6 were killer-sensitive strains. Additionally, 8 strains were found to have neutral character. All *Debaryomyces hansenii*, *Pichia anomala*, *Clavispora lusitaniae* and *Kloeckera apiculata* strains were found as killer. No sensitive strain was found among the tested isolates. In conclusion, killer behavior of the endogenic yeast strains of Gemlik cultivar olives of Akhisar and Iznik regions was found as common characteristics.



**FEMS-0985**

**Food microbiology**

**INVESTIGATION OF BRUCELLA SPP. AND BRUCELLA DNA IN RAW MILK  
OBTAINED FROM TRAKYA REGION, EDIRNE, TURKEY**

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**Background**

Brucellosis is an important zoonotic disease transmitted to human by consuming contaminated milk and milk products. The prevention from Brucellosis is provided by live-attenuated vaccines for animals. Although Trakya is the pilot region in Turkey, for vaccination of animals, Brucellosis is endemoepidemic in this region. Thus, many dairy companies supply their milk from the cows grown in this region; screening of milks for *Brucella* contamination becomes very important.

**Objectives**

Our aim was to investigate *Brucella spp.* in milk, procured from Edirne and compare the conventional and molecular methods in terms of screening *Brucella spp.* in milk.

**Methods**

Milk samples from 99 cows at 12 different barns in 5 different villages of Edirne were collected. Bacteriological analyses and Q-PCR were applied to all samples. For samples that were culture negative and Q-PCR positive, a Q-PCR based method was evolved to differentiate the virulent and the vaccine strains.

**Conclusions**

In 2 of the 99 milk samples, *Brucella spp.* was isolated by bacteriological methods. After Q-PCR, *B. ovis* and *B. melitensis* were not detected, however, in 16 of the 99 samples *B. abortus* was detected. After the Q-PCR based method evolved to differentiate virulent and vaccine strains, only the 2 bacteriologically positive samples were detected to be positive.

As a result, it is reported that; 2.02% of the samples were detected to contain *Brucella*, both with the bacteriological method and PCR. It is also determined that, to obtain true positive results in *Brucella spp.* screening studies for milk, differentiating the virulent and vaccine strain should not be disregarded.

**FEMS-2039**

**Food microbiology**

**PRODUCTION OF ACE-INHIBITORY PEPTIDES BY LACTIC ACID BACTERIA ISOLATED FROM TRADITIONAL GREEK DAIRY PRODUCTS.**

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**Background**

Fermented dairy products are generally considered to be beneficial for human health as they contain live lactic acid bacteria frequently exhibiting biofunctional features. Here we present 55 lactic acid bacteria isolated from three traditional Greek yogurt samples and five fermented milk samples where their ability to produce bioactive peptides with inhibitory activity against the angiotensin-converting enzyme (ACE) was evaluated.

**Objectives**

The aim of this study is the potential of several lactic acid bacteria strains to be used as health promoting starter cultures with interesting technological features.

**Methods**

Strain typing was performed by rep-PCR, while 16S rDNA sequencing was used for the identification at the species level. The angiotensin-converting enzyme inhibitory (ACE-I) activity of the isolates was evaluated using a spectrophotometric assay with N-Hippuryl-His-Leu hydrate as substrate. Finally, a semi-preparative HPLC analysis was conducted using an ACE-5C18 column and positive fractions were subjected to MS analysis.

**Conclusions**

During this work 55 microorganisms have been isolated from traditional Greek yogurt and fermented milk samples. Strain typing by rep-PCR showed that the 33 isolated bacilli corresponded to 10 and the 22 cocci to 12 strain groups, respectively. The

strains did not show neither strong acidification capacity, nor peptidolytic activity and citrate metabolism, so their use as starter cultures does not seem suitable. Among them, many strains possessed interesting technological features as proteolysis and lipolysis and few exhibited high ACE-I activity. Purification of the ACE-I peptides was performed by RP-HPLC. These results indicate their potential use as adjunct or probiotic cultures.

**FEMS-3058**

**Food microbiology**

## **EVALUATION OF CINNAMON AND MARJORAM ESSENTIAL OILS FOR THE CONTROL OF MYCOTOXIGENIC FUSARIUM SPECIES**

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### **Background**

*F. culmorum*, and *F. graminearum* are the most common causes of Fusarium ear blight of corn, wheat and other grains. Both of them produce the trichothecenes mycotoxins and *F. graminearum* also produces zearalenone.

### **Objectives**

The aim of this study was to evaluate the efficacy of cinnamon and marjoram essential oil (EOs) to control the growth of Fusarium species in vitro and to observe the changes of morphological characteristics of the fungi by SEM images after treatment with the oils.

### **Methods**

Colony growth inhibition effect of the EO vapors was investigated on the *Fusarium* species by the reversed Petri dish method using 0.070 mg/l, 0.14 mg/l, 0.28 mg/l and 0.56 mg/l EO/dish. The antifungal effect was measured by determination of growth-rate (mm/day) and antifungal index (%). MIC (minimal inhibitory concentration) and MFC (minimal fungicidal concentration) values were determined by agar dilution method. Morphological characteristics of the hyphae and micro conidia of *F. culmorum* were observed using a scanning electron microscope.

### **Conclusions**

Cinnamon EO vapor at 0.28 mg/l concentration caused total growth inhibition of all investigated species. Using marjoram EO vapor total inhibition was not detected, but growth-rates and colony diameters were reduced in each case. Agar dilution method was more effective to inhibit the growth of *F. culmorum* (MIC value =2.5 mg/ml). SEM imaging of *F. culmorum* showed that EO treated hyphae were thinner. Rupture of the cell wall and leakage of the cytoplasm contents were also observed. This study showed that EOs have potential antifungal effect against mycotoxigenic Fusarium species.



**FEMS-1546**

**Food microbiology**

## **CHROMOHALOBACTER AS A CAUSATIVE AGENT OF THE PUTRESINE PRODUCTION DURING THE FERMENTATION OF GANJANG, KOREAN TRADITIONAL SOY SAUCE**

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### **Background**

In fermented foods, the presence of biogenic amines (BAs), which were produced by microbial decarboxylase activity of microorganism, might serve as a useful indicator of food poisoning. Although BAs are also frequently found in ganjang, representative Korean traditional soy-sauce, there is no information about the BAs-producing microorganism in ganjang.

### **Objectives**

In this study, to investigate microorganism responsible for BAs production in ganjang, metabolite and bacterial community analysis were performed during entire fermentation process of ganjang.

### **Methods**

Ganjang with a NaCl concentration of approximately 18% (w/v) was prepared in one batch using traditional manufacturing method. The same amounts of ganjang were periodically sampled and their pH, NaCl concentration, and cell number were measured. <sup>1</sup>H NMR and PCR-DGGE were applied for analysis of metabolites and bacterial succession during the ganjang fermentation, respectively. In order to investigate the correlations among the ganjang samples, bacterial communities, and metabolites, a multivariate statistical redundancy analysis was performed using the vegan package of the R programming environment.

### **Conclusions**

Metabolite profiling and bacterial community analysis using a <sup>1</sup>H NMR and PCR-DGGE showed that a large amount of putrescine detected after 74 days of fermentation, which time begins to predominate the members of *Chromohalobacter*. A statistical analysis based on metabolite profiling and bacterial succession data clearly shows that members of *Chromohalobacter* are involved in the production of putrescine during ganjang fermentation. This study will allow for the successful understanding of the BAs-producing microorganism in ganjang.



**FEMS-2780**

**Food microbiology**

**CHARACTERISTICS OF CONTAMINANT MICROBES IN COMPOUND FEEDS  
AND THE GROWTH INHIBITION EFFECT BY PROBIOTIC B. SUBTILIS**

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**Background**

During long term storage of animal compound feed, contaminated microbes can grow with providing a problem of feed safety.

**Objectives**

The aim of this study was to investigate contaminant microbes and the effects of probiotic B. subtilis to inhibit them in animal feeds.

**Methods**

During storage of feed at room temperature, the changes of pH and titrable acidity were monitored with total microbial counts. Bacterial and fungal strains were isolated and tested for antibiotics resistance. Contaminated microbes were further identified by the sequencing of 16S and 18S rDNAs.

Probiotic B. subtilis was inoculated into both non-sterile and sterile feeds and tested for the growth inhibition of contaminated microbes during storage of feed at room temperature with pH change and probiotic viability.

**Conclusions**

Most contaminated microbes were originated from soil and plants. Sterile and non-sterile compound feeds displayed a different pattern of pH change. B. subtilis showed a normal growth and inhibited the cell growth of contaminant microbes.

**FEMS-1513**

**Food microbiology**

**EVALUATION OF MICROBIAL CONTAMINATION LEVELS AT THE PRE- AND POST-GERMINATION STAGES ON SPROUTS.**

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**Background**

The consumption of raw sprouted seeds has led to a large number of outbreaks of foodborne illness in a great number of countries.

**Objectives**

The objective of this study was to investigate and evaluate microbial contamination levels of several kinds of sprouts in pre- and post-germination stage.

**Methods**

Fourteen kind of sprout seeds were purchased and analyzed. Quantitative analyses comprised aerobic plate counts (APCs) and the measurement of coliforms and *Bacillus cereus* levels, whereas qualitative analyses involved assessing the levels of *Escherichia coli* and major foodborne pathogens (*E. coli* O157:H7, *Listeria monocytogenes*, *Salmonella* spp. and *Staphylococcus aureus*).

**Conclusions**

The APCs ( $2.3\text{--}4.07 \log \text{CFU g}^{-1}$ ) for sprouts seeds increased by approximately 3-4  $\log \text{CFU g}^{-1}$  during germinating, reaching  $5.61\text{--}8.94 \log \text{CFU g}^{-1}$ . Similarly, increasing trends were noted in the level of *B. cereus* (N.D.- $2.62 \log \text{CFU g}^{-1}$  at the seed stage, increasing to N.D.- $3.14 \log \text{CFU g}^{-1}$  by the germinated stage). *E. coli*, *E. coli* O157:H7, *Salmonella* spp. and *L. monocytogenes* were not detected in the sprouts analyzed in this study. The present study provides comprehensive information regarding the microbiological safety of seeds and sprouts during manufacturing.

**FEMS-2198**

**Food microbiology**

## **ANTIOXIDANT ACTIVITY OF FILAMENTOUS CYANOBACTERIAL STRAINS**

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### **Background**

Antioxidants are compounds with role in food preservation, as well as in the prevention and treatment of some degenerative diseases. Despite their high amounts in plants, there is a growing interest for less conventional sources. Cyanobacteria, as a particular producers of bioactive compounds, represent an important source of antioxidants, which in terms of chemical structure include diverse compounds.

### **Objectives**

In the present study, the antioxidant capacity of three filamentous cyanobacterial strains belonging to the *Anabaena*, *Nostoc* and *Spirulina* genera have been tested. Furthermore, the content of C-phycocyanin, a pigment known as antioxidant compound was determined.

### **Methods**

The antioxidant capacity was tested using DPPH and FRAP assays. In DPPH assay, strains *Spirulina* S1 and *Anabaena* C5 showed similar IC<sub>50</sub> values of 118.47 µg/ml and 120.26 µg/ml respectively. In FRAP assay, the highest antioxidant activity was observed in *Spirulina* strain S1, ranging from 452.41 to 637.38 mg of ascorbic acid (AA) equivalent/gram of dry extract. The lowest antioxidant capacity expressed strain *Nostoc* 2S9B in both assays (IC<sub>25</sub> of 21.93 µg/ml, and 142.21-350.18 mg of AA equivalent/gram of dry extract).

The C-phycocyanin content was determined spectrophotometrically. The highest value of 38.63 µg/ml was detected in strain *Spirulina* S1, while lower contents were obtained in strains *Nostoc* 2S9B and *Anabaena* C5 (6.57 µg/ml and 9.34 µg/ml respectively).

### **Conclusions**

Since *Spirulina* strain S1 has expressed the highest antioxidant capacity in both assays, it could be further investigated as a potential source of antioxidants. High C-phycocyanin content found in this strain could be associated with its antioxidant activity.

**FEMS-0979**

**Food microbiology**

## **IDENTIFICATION OF BIOLOGICAL HAZARDS IN SPICES AND HERBS**

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### **Background**

Securing the food chains from primary production to consumer-ready food against major deliberate, accidental or natural contaminations is directly related to the safety of food products. Spices and herbs are natural products of almost all processed food that can be contaminated with several microorganisms and toxins. The identification of those condiments would be difficult, as consumers and experts often focus on major food ingredients instead of minor components. Securing of spices and herbs commodity chains is the key issue within the EU FP7 project SPICED.

### **Objectives**

Characterization of the heterogeneous matrices and their respective production and supply chains in context with relevant biological hazards and the improvement of the knowledge on biological hazard properties as well as on-site and high throughput diagnostic methods for appropriate detection are main tasks.

### **Methods**

Within the SPICED project we mainly focusses on the toxins Ricin, which is the toxic component from castor beans of *Ricinus communis*, and the Enterotoxin-type B produced by the gram-positive bacteria *Staphylococcus aureus* (SEB). Their qualitative and quantitative detection in spices and herbs including the development of sample preparation methods were performed. For this purpose different types of spices and herbs were spiked with the toxins, stored at room temperature and analyzed e.g. with immunological based methods. Fieldable technologies and methods for the rapid on-site detection of toxins in the different were also examined.

### **Conclusions**

The intention is to present the recent results on the detection and sample preparation of the toxins from the spices and herbs with laboratory and field methods.



**FEMS-0737**

**Food microbiology**

**EFFECT OF THERMALLY PROCESSED RICE KOJI EXTRACTS ON  
CHRONOLOGICAL LIFE SPAN OF SACCHAROMYCES CEREVISIAE ON HIGH-  
SUGAR FERMENTATION**

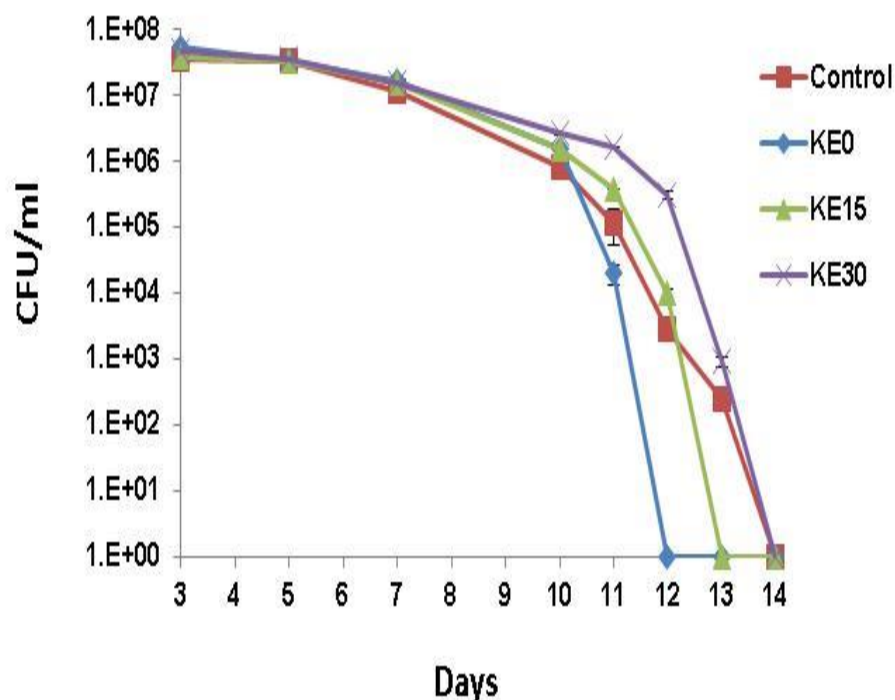
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**Background**

Yeast cell growth and viability on high ethanol concentrations become limiting factors in fermentation productivity and ethanol yield. That is why measures to improve ethanol tolerance in yeast have been expected.

**Objectives**



**Fig. Transition of yeast populations (*S. cerevisiae* K-701) during fermentation in YNB20 medium with or without *koji* extracts (KE).**

**Different heat-processed (0, 15, and 30 min at 121°C) *koji* extracts were added at a final concentration of 10% (V/V). Fermentation was performed with the inoculation of precultures grown in YNB medium at 0.01 volume to each medium at 30°C.**

This study focused on *koji* as a material for sake brewing which provides nutrients for yeast cell growth. Our goal is to develop strategies for extending the life span of yeast by revealing the influence of heat-processed *koji* extracts on the survival of yeast under ethanol stress.

## Methods

Rice *koji* extracts were prepared by water extracts of dried rice *koji* heated at 60°C for 5 hrs. The *koji* extract was autoclaved for different times at 121°C. The availability of thermally processed rice *koji* extracts was investigated by analysis of ethanol tolerance and survivals on high-sugar fermentation in *S. cerevisiae* K-701 while making a comparison between heated and non-heated *koji* extracts.

## Conclusions



Addition of heat-processed *koji* extracts to high-sugar medium led to an improvement of chronological life span in *S. cerevisiae* K-701. The longer the heating time of *koji* extracts was, the higher the population of *S. cerevisiae* was during the late stationary phase. The Maillard reaction products which occurred in the heat-processed *koji* extracts had an explicit effect on the survivals of yeast under lethal ethanol stress. These show that heat-processed rice *koji* extracts were served as protectants of both ethanol and oxidative stresses when yeast cells became more oxidized in the stationary phase at higher ethanol concentrations.

**FEMS-1660**

**Food microbiology**

**STUDY FOR INCREASING THE ANTIOXIDANT COMPOUNDS BY BACTERIA ISOLATED FROM FERMENTED FOOD**

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**Introduction**

Oxidative stress arising from an imbalance in the human antioxidant status, reactive oxygen species vs. defense and repair mechanisms, is mainly responsible for the development of pathological disease. Phenolic compounds in safflower seeds are receiving much attention as potential therapeutic agents against several pathological diseases. The antioxidant properties of safflower seeds is matairesinol 4'-o- $\beta$ -D-glucose, 8'-hydroxyarctigenin 4'-o- $\beta$ -D-glucose, matairesinol, 8'-hydroxyarctigenin, N-feruloylserotonin 5-o- $\beta$ -D glucoside, N-(p-coumaroyl)-serotonin-5-o- $\beta$ -D-glucoside, N-feruloylserotonin, N-(p-coumaroyl)serotonin, luteolin 7-o- $\beta$ -D-glucoside, luteolin, acacetin 7-o- $\beta$ -D-glucuronide, and acacetin.

**Objective.**

In our experiment, bacteria isolated from ginger fermented food were used to make phenolic compound from aglycon. It makes to increase antioxidant activity of safflower seeds.

**Methods**

Fermented samples were got from one of the member of Korea royal cuisine institute. Fermented sample was inoculated in tryptic soy broth media and incubated 28°C for 48h. After bacteria grow, bacteria were centrifuged 6000rpm at 4°C for 20 min. Bacteria were dissolved 60ml mineral salt media (MSM) (20g/L) and mixed with 30g safflower seed. Each bottle was incubated 28°C for one week.

For phenolic compound extraction, each safflower seed mixed with bacteria was sonicated in hexane for 30min twice and filtered. The defatted seed residue was extracted twice with 70% EtOH under sonication, filtered and evaporated under reduced pressure. The extract was used for HPLC and GC-MS and DPPH activity.

**Conclusion**

This study was to evaluate the antioxidant activity in safflower seeds after fermentation. Bacteria that improved antioxidant activity in safflower seeds after fermentation was identified Sphingomons sp using 16s rRNA sequencing. Safflower seed was improved antioxidant activity from 7.11% to 51% after 5 days. In HPLC analysis, Major peak of safflower seed extracts after fermentation was changed after 5 days under aerobic condition at 30°C. Furthermore analysis, we will conduct to investigate which compound in the safflower seed will be changed to phenolic compound for increasing antioxidant activity.



**FEMS-1512**

**Food microbiology**

## **EFFECTS OF SEED TREATMENT BY COLD ATMOSPHERIC PLASMA ON CUCUMBER SEEDLING GROWTH**

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### **Background**

Cold atmospheric plasma can have different effects on seed germination and seedling growth depending on the plasma frequency, the power and its environmental conditions.

### **Objectives**

Cold plasmas are already well-known for their improvement effect on plant growth. However, the mechanisms of this ability are unclear.

### **Methods**

Non-thermal atmospheric-pressure DBD plasma in He was applied with different exposure times on cucumber seed. Changes in the seed surface with different exposure time were examined by scanning electron microscope (SEM). Direct and indirect influence on cucumber seed of the cold plasma is compared in terms of seedling growth.

### **Conclusions**

Germination and early seedling growth of cucumber were improved by the plasma treatment for 6 second. In contrast, the seeds exposed for 10 minutes were not germinated. In 6 sec treatment and no treatment control, gold nanoparticles were observed along the cell wall. There were no particles observed in the 10 min treatment. It suggests that no tissue damage is observed visually or microscopically following 6 sec and control, but the rough surface of seeds were smooth and damaged at long exposure time (10 min). We observed that the indirect method had more enhanced effect on growth than direct method. This infers to an important conclusion that the effect of plasma on improved growth of seedlings is due to various plasma components that can exist inside or outside the plasma region not mechanical power such as an abrasion of seed surface.

**FEMS-1648**

**Food microbiology**

## **METABOLICALLY ACTIVE BACTERIA IN LYMPHATIC TISSUES OF PIGS AND ITS SPREAD DURING SLAUGHTER**

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### **Background**

The occurrence of potential food-borne pathogenic organisms in lymphatic tissues of farm animals has practical relevance for carcass contamination. A transmission of pathogens, which can occur from harvesting until the last step in the meat-processing chain, remains the preeminent difficulty in slaughter house hygiene.

### **Objectives**

The aims of this study were i.) to evaluate the contamination degree of porcine cervical musculature during slaughter, ii.) to compare the bacterial microbial community detected by DNA-based amplicon sequencing with RNA-based amplicon sequencing and thus measuring the fraction of metabolically active bacteria in lymphatic tissues.

### **Methods**

Mandibular lymph nodes, tonsils and scrapings from cervical musculature were sampled from eight healthy slaughter pigs derived from different farmers. RNA was isolated from lymph node cortex regions and tonsils (n=16) and DNA was isolated from cervical musculature (n=8). Total RNA was transcribed into cDNA and all samples were sequenced with Roche/454 using 16S rRNA gene amplicons.

### **Conclusions**

Organisms relevant for food safety, e.g. *Erysipelothrix*, *Listeria*, *Escherichia* and *Pseudomonas*, could be detected in lymphatic tissues. RNA-based amplicon sequencing revealed a highly diverse, metabolically active bacterial microbiome in mandibular lymph nodes and tonsils; consisting of 348 and 390 operational taxonomic units (OTUs) respectively (30% shared OTUs). The functional metagenome prediction (PICRUSt) and subsequent assignments of orthologues to COG categories revealed amino acid transport and metabolism- related orthologues being increased in lymph nodes compared to tonsils. Both, mandibular lymph nodes and tonsils of slaughter pigs harbor a replication-competent bacterial microbiome indicating a possible contamination source during and after slaughter.

**FEMS-2106**

**Food microbiology**

## **ISOLATION AND CHARACTERIZATION OF THE MICROFLORA PRESENT IN A MINIMALLY PROCESSED VEGETABLES PLANT**

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### **Background**

Food safety is an area of growing concern due to the increasing demand for microbiologically safe products. Additionally, disinfection techniques must evolve to cope with the development of antimicrobial resistance. An efficient disinfection strategy must consider the type of microbial contaminant. Therefore, the knowledge on the microorganisms present in an industrial process is crucial to define the best strategy for their control.

### **Objectives**

The aims of the present work were to isolate and characterize the resident microflora present in a minimally processed vegetables (MPV) plant.

### **Methods**

The microorganisms were isolated from a MPV plant process surfaces by cotton swabbing, from the air and from the vegetable surfaces. The isolates were identified by 16S rRNA gene sequencing with 4 primers: 27F, 518F, 800R e 1492R. The isolates were also characterized for their production of proteases, gelatinases and siderophores, quorum-sensing inhibition and biofilm formation ability.

### **Conclusions**

From 50 different isolates 46% belong to the *Pseudomonas* genera and 22 were from different species. Some microorganisms persisted along the food chain, which means that the cleaning process should be targeted for the more resistant microorganisms. Most of the microbial isolates are capable of producing virulence and food spoilage molecules. Moreover, *Pseudomonas* were the genera with a higher biofilm formation ability, being the predominant microflora and showing recalcitrant properties along the process chain.

**FEMS-1615**

**Food microbiology**

**TECHNOLOGY, BIOCHEMICAL AND MICROBIOLOGICAL FEATURES  
INFLUENCING THE CONCENTRATION OF BIOGENIC AMINES OF TWENTY  
APULIAN AND SICILIAN (SOUTHERN ITALY) CHEESES**

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**Background**

Biogenic amines (BA) are low-molecular nitrogenous organic bases, which are formed in foodstuffs by microbial decarboxylation of the precursor amino acids and are potentially toxic to human health. Cheeses are among the foods most commonly associated with the presence of BA (1). Concentration of BA results from a balance between amino acid decarboxylating and amine oxidising activities.

**Objectives**

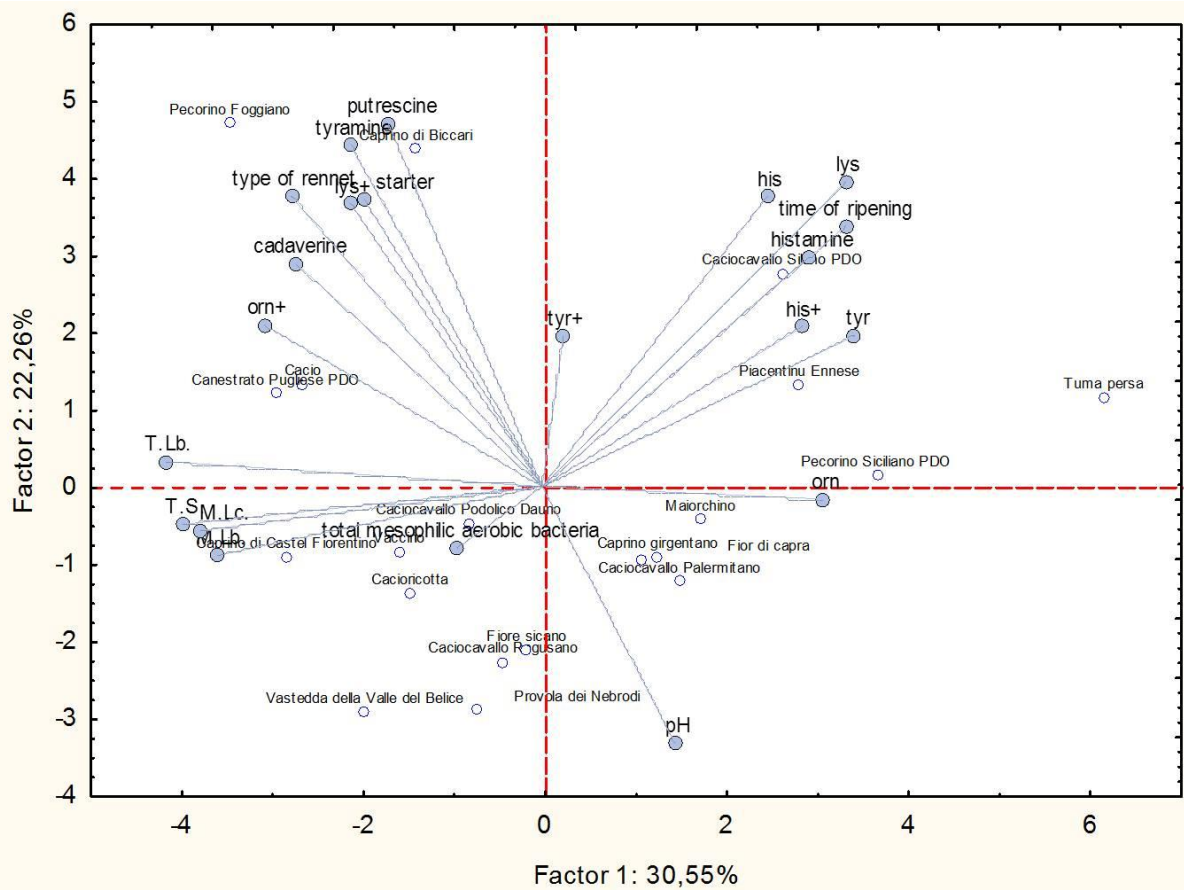
To correlate the content of BA of twenty traditional Apulian or Sicilian (Southern Italy) cheeses with several technology and microbiological features such as time of ripening, pH, concentration of precursor free amino acids (FAA), and occurrence of decarboxylase-positive lactic acid bacteria.

**Methods**

Cheeses were analysed through plate counting and HPLC, and lactic acid bacteria isolated from cheeses were assayed (by decarboxylase medium) for their capacity to generate BA. Principal Component Analysis was performed to find the effect of different parameters on the distribution of the cheeses.

**Conclusions**

Although short-ripened cheeses did not show significant BA concentrations, the only BA showing high positive correlation with time of ripening was histamine. Concentration of histidine and, especially, percentage of histidine-decarboxylase bacteria presumably affected histamine concentration. High pH values were negatively correlated to the concentration of tyramine, putrescine, and cadaverine (Fig. 1). Fifty percent of the cheeses contained at least one BA at potentially toxic concentrations. Unambiguous and ever-valid relations among parameters and BA are difficult to determine, because BA are the result of combined and varied factors.





**FEMS-1791**

**Food microbiology**

## **DYNAMICS OF BACTERIAL COMMUNITIES DURING THE RIPENING PROCESS OF CROATIAN RAW EWE'S MILK CHEESE**

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### **Background**

Croatian raw ewe's milk cheeses are hard cheeses that are produced by traditional techniques without pasteurization and application of starter cultures, and they are characterized by an aging process of 90-120 days. This aging time is rather long and the low pH and water activity ( $a_w$ ) of ripened cheese usually does not support the growth of pathogens. However, due to the commercial pressure, the ripening time has been shortened and it has become market practice to sell the cheese as soon as possible.

### **Objectives**

A close monitoring of bacterial communities and a reliable identification of indigenous microflora is crucial in order to maintain the quality and safety of artisan cheese.

### **Methods**

Cheese samples of three cheese types (0, 45 and 90 days of ripening) were collected from 6 cheese makers. Total DNA was extracted and microbial diversity was investigated based on fingerprinting in combination with next generation sequencing of 16S rRNA gene amplicons.

### **Conclusions**

Overall up to 213 OTU<sub>97</sub> could be assigned. Twenty of the major OTUs were present in all samples and include mostly LAB, mainly *Lactococcus* and *Enterococcus* species. Abundance and diversity of these genera differed to a large extent between the 3 investigated cheese types and in response to the ripening process. Also a large number of non LAB genera could be identified based on phylogenetic alignments including mainly *Enterobacteriaceae* and *Staphylococcaceae*. Some species belonging to these two families could be clearly assigned to species which are known as potential human pathogens. However, during cheese ripening their abundance was reduced.

**FEMS-1809**

**Food microbiology**

**TRACKING OF PATHOGENIC AND BENEFICIAL MICROFLORA FROM WILD BOAR MEAT SAUSAGES TRADITIONALLY PRODUCED IN CROATIA**

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**Background**

Sausages from game meat produced by traditional procedures by small scale producers in Croatia do not include the application of starter cultures. Such artisan products are increasingly appreciated because of their sensory properties and authenticity, although considerations regarding their microbiological safety are existent.

**Objectives**

An in depth analysis and characterization of the indigenous microflora of traditionally produced wild boar meat sausages from Croatia is needed in order to estimate microbiological hazards and to preserve an indigenous microbial pool.

**Methods**

Traditionally produced wild boar sausages were collected from three farms during ripening (0, 2, 4, 7, 10, 20 and 40 days) and analyzed for spoilage and pathogenic microflora (*L. monocytogenes*, *Salmonella* spp., total *Enterobacteriaceae*, coliforms, yeasts and molds, *B. cereus* group and coagulase-positive staphylococci) as well as for beneficial microbes e.g. lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS). Genomic DNA was extracted from 720 LAB and CNS isolates. After RAPD and rep-PCR based grouping identification was done by PCR and partly 16S rRNA gene sequencing.

**Conclusions**

A remarkable diversity was found among the investigated LAB and CNS populations of which several isolates relevant for sausage ripening and product quality were identified as e.g. *Lactobacillus sakei*, *Leuconostoc mesenteroides*, *Staphylococcus xylosus*, *Staphylococcus saprophyticus* and *Staphylococcus warneri*. Although some spoilage and pathogenic microflora were present at the initial stages of sausages' ripening, they were absent in the ripened "ready to eat" sausage.

**FEMS-1595**

**Food microbiology**

## **REDUCING THE RISK OF BABY LEAF SURFACE BACTERIA WITH IRRIGATION RESTRICTIONS**

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### **Background**

Baby leaf crops like Spinach (*Spinacia oleracea*), Chard (*Beta vulgaris*) and Rocket (*Diplotaxis tenuifolia*) are grown on huge open space flat fields in Sweden. It is hard to stop birds and wild animals to visit the field with related risks of E. coli infections. But as a grower, one can make sure the irrigation water doesn't act as a bacteria vector.

### **Objectives**

The main objective of this study was to reduce the risk of E. coli bacteria reaching baby leaf crops during crop growth in field, thereby reducing the risk of food borne diseases further down the delivery chain.

### **Methods**

Leaves were grown with more or less constant optimal wet conditions in the root zone (irrigation water given up to field capacity every other day) or with restricted irrigation (drought conditions) in the final part of the growth period. 24 hours prior to harvest plants were sprayed with a known quantity of E. coli. Harvested leaves were put in a stomacher and the leaf rinsates plated on agar and the colonies counted and compared to non-sprayed leaves.

### **Conclusions**

Irrigation restriction only had minor impact on E. coli colonization on the leaf surface. The yield and quality was significantly reduced and the content of bioactive compounds affected. The main conclusion is that drought stress during the final part of baby leaf production is not to recommend and as a way to reduce the risk of food borne diseases caused by baby leaf salads.



**FEMS-2542**

**Food microbiology**

## **IMPROVED DETECTION OF ESCHERICHIA COLI AND COLIFORM BACTERIA BY MULTIPLEX PCR**

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### **Background**

The presence of coliform bacteria is routinely assessed to establish the microbiological safety of water supplies and raw or processed foods. Coliforms are a group of lactose-fermenting *Enterobacteriaceae*, which most likely acquired the *lacZ* gene by horizontal transfer and therefore constitute a polyphyletic group. Among this group of bacteria is *Escherichia coli*, the pathogen that is most frequently associated with foodborne disease outbreaks and is often identified by  $\beta$ -glucuronidase enzymatic activity or by the redundant detection of *uidA* by PCR.

### **Objectives**

Because a significant fraction of essential *E. coli* genes are preserved throughout the bacterial kingdom, alternative oligonucleotide primers for specific *E. coli* detection are not easily identified.

### **Methods**

Here two strategies were used to design oligonucleotide primers with differing levels of specificity for the simultaneous detection of total coliforms and *E. coli* by multiplex PCR. A *lacZ* sequence and an orphan gene were chosen as targets for amplification.

### **Conclusions**

A comparison with previously described primers indicates an increase in identification efficacy when tested with laboratory collection and lactose-fermenting strains isolated from dairy samples. While *lacZ* amplicons were found in a wide range of lactose-fermenting strains, amplification using the selected orphan gene was highly specific for *E. coli*. Additionally, the detection of this target sequence is non-redundant with enzymatic methods.

**FEMS-2683**

**Food microbiology**

**EFFECT OF OXYGEN CONCENTRATION AND GAS PRESSURE ON THE GROWTH OF ARTHROSPIRA SP. PCC 8005**

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**Background**

The MELiSSA project of the European Space Agency, ESA, aims to create a bio-regenerative life support system for long term space travels. One of its bioreactors contains *Arthrospira* sp. PCC 8005, an edible cyanobacteria that can remove CO<sub>2</sub> and provide O<sub>2</sub>. Normal operating conditions will likely enhance oxygen concentration and gas pressure buildup.

**Objectives**

The objective of this study is to elucidate the impact of oxygen concentration and gas pressure on the duration of the lag phase, the specific growth rate and the maximum number of attained generations in a batch culture of *Arthrospira* sp. PCC 8005.

**Methods**

Cultures of *Arthrospira* sp. PCC 8005 were grown in 100 ml closed septum vials with Zarrouk-UBP medium, at 30 °C with 32 µE m<sup>-2</sup> s<sup>-1</sup> of irradiance and shaken at 130 rpm. Growth was monitored measuring OD<sub>750nm</sub>. Produced oxygen was accumulated. Oxygen tests were done with an initial 100% oxygen gas phase, in comparison to 100% argon, both at initial 1 bar. Pressure tests were done at 1, 2 and 3 bars of starting absolute pressure in 100% argon. Growth parameters were calculated using a modified Gompertz function. Statistical analysis was performed with 95% confidence interval T-Test.

**Conclusions**

The oxygen concentration has only a limited but statistically significant effect on the growth rate. Growth tests at elevated pressures of up to 3 bar had no effect on neither growth parameter. Test results at elevated pressures with 100% oxygen pressures, are expected to prove and further quantify the inhibitory effect of oxygen on the growth.

**FEMS-2587**

**Food microbiology**

**DETECTION OF ITURIN AND FENGYCIN BY MALDI-TOF IN FOOD MATRIX OF PUBA, A STAPLE BRAZILIAN FERMENTED FOOD**

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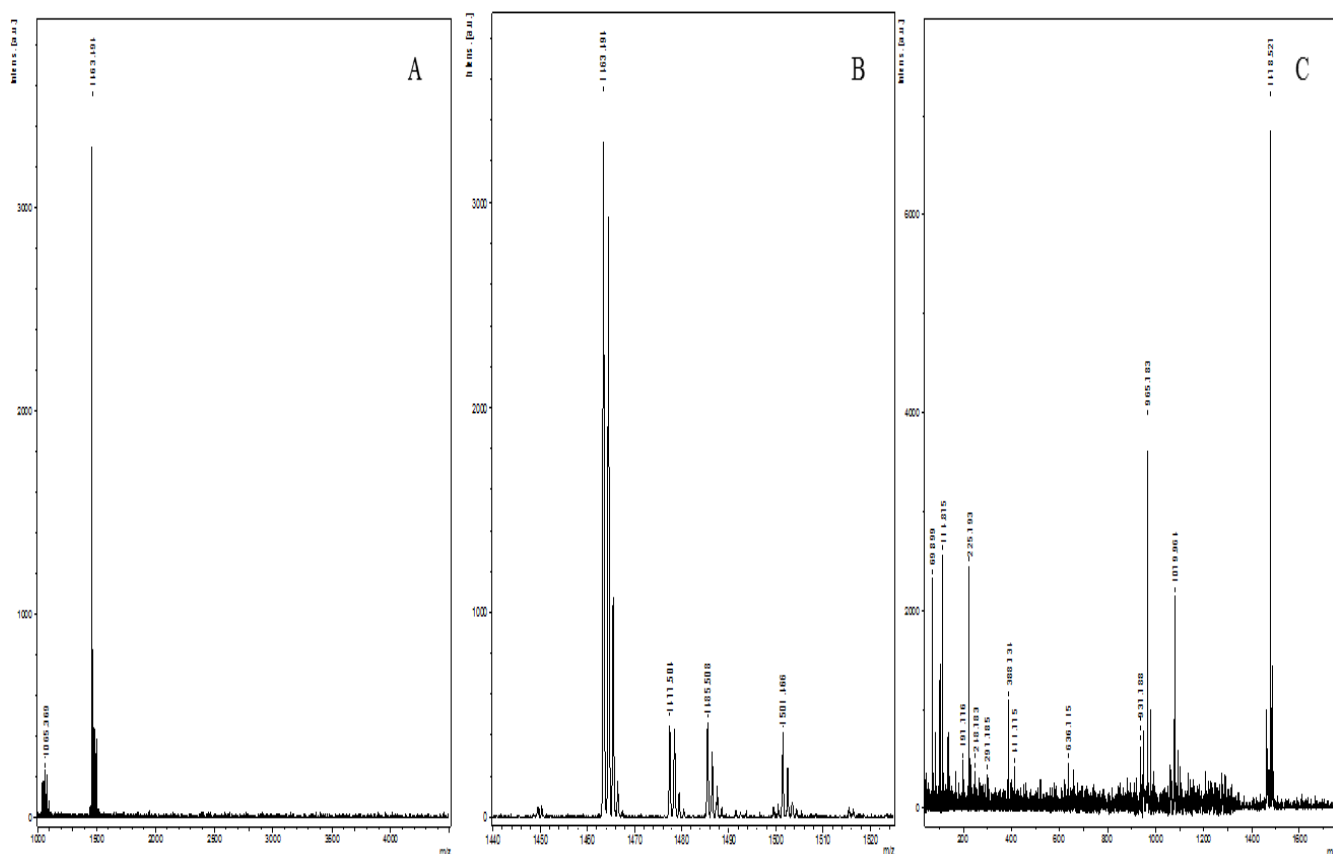
**Background**

*Puba* or *carimã* is a Brazilian staple food obtained by spontaneous submerged fermentation of cassava (*Manihot esculenta*, Crantz) roots. Traditional fermentation of cassava is predominantly performed by lactic acid bacteria, but yeasts and *Bacillus* spp. were also found in our sample.

**Objectives**

To investigate the production of lipopeptides by *Bacillus* sp. P5 isolated from *puba* in the culture medium and in *puba* matrix by MALDI-TOF.

**Methods**



In a previous study, 16S rRNA of *Bacillus* sp. P5, isolated from *puba*, was sequenced. The phylogenetic analyses showed recovery in a node with 92% of support with *B. amyloliquefaciens*. The culture supernatant was subjected to extraction with n-butanol (Landy medium) purification by reversed-phase HPLC (C18 column) and MALDI-TOF mass spectrometry analysis. Surfactins, iturins and fengycins were detected. To investigate the production of lipopeptides by *Bacillus* sp. P5 isolated from *puba* in the culture medium and in *puba* matrix by MALDI-TOF. An aqueous suspension of *puba* was extracted with butanol and concentrated in Zip Tip® C18. Molecular mass was determined by MALDI-TOF. Data were acquired in the mass range m/z 1,000-4,000 for the butanol extract. The results are shown in Figure 1. Two clusters were found: iturin and fengycin (Fgy) (1A). Expansion of the spectrogram of Fgy indicated protonated ion and sodium and potassium adducts (1B), and in (C) MS/MS spectrum of precursor ion of m/z 1478.5 (Fgy C<sub>17</sub>) which generated fingerprints of fgy A.

## Conclusions

In conclusion, *puba* is manufactured in an unhygienic environment, and iturin and fengycin exhibit biocontrol properties that are probably important for the safety of this product.



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**FEMS-0519**

**Food microbiology**

**BACTERIAL ASSESSMENT OF FOOD - HANDLERS IN SARI CITY AND ITS SUBURB , MAZANDARAN PROVINCE, NORTH OF IRAN.**

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**Background**

Diarrheal diseases, mostly caused by food borne or waterborne microbial pathogens, are leading causes of illness and deaths in developing countries, killing an estimated 1.9 million people annually at the global level.

**Objectives**

This study was designed to determine the prevalence of carriers of pathogenic bacteria among the food-handlers employed at food service facilities in Sari city and its suburbs, Mazandaran Province, Iran

**Methods**

Of the total subjects examined, 62.2 % were found to be carriers of some pathogenic bacteria.

The pathogens isolated and identified were the bacteria *Staphylococcus aureus*, *shigella sonnei* and *pseudomonas aeroginosa*.

Fast food makers were the greatest prevalence of bacteria in respect of their fingernail contents (86.6 %,  $p=0.04$ ) followed by butchers (76.4%), storekeepers (73%), bakers (58.3%) and restaurant workers (51.5 %). *Staphylococcus aureus* was the predominant bacteria isolated from nail-washing samples (45.9%), followed by *Escherichia coli* (28.4%), *coliform* (18.2%) and *Pseudomonas aeroginosa* (6.5%),

Illiterate food-handlers had the highest percentage of positive cultures from nail-washing specimens (86.8%,  $p= 0.0005$ ) and the lowest rate of infestation was among university educated subjects (33.3 %).

The rate of of *Staphylococcus aureus* nasal carrier among food-handlers was 18.6 % with the highest rate among store-keepers (31.7%) followed by bakers (24.3%), restaurant workers (19.5%), butchers (14.6%) and fast-food makers (9.7%). *Shigella sonnei* were isolated from stool samples of two food-handlers (0.9%).

**Conclusions**

The finding emphasized the importance of food-handlers as potential sources of infections. A medical check –up program with health education could improve worker's health status.



**FEMS-1135**

**Food microbiology**

## **ISOLATION AND MOLECULAR IDENTIFICATION OF LACTIC ACID BACTERIA FROM DONKEY MILK**

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### **Background**

Lactic acid bacteria (LAB) are living organisms that play a major role in many food and dairy products as a probiotics. Donkey's milk is being used as a source of human nutrition, especially for infants with allergy to cow milk proteins, due to its antimicrobial components, many defense factors and probiotic properties. Few studies have so far dealt with the microbiota in donkey's milk, specifically lactic acid bacteria (LAB) diversity.

### **Objectives**

This study was undertaken to identify the microfora of LAB in donkey's milk in IRAN.

### **Methods**

The culture-dependent microbial and 16S rRNA sequencing techniques were used to identify lactic acid bacteria species in donkey milk. A total of 250 LABs from donkey milk were assessed by culture-dependent microbial tests, including culture at different temperatures, pH and Multiple antibiotic resistance indexing.

### **Conclusions**

*Enterococcus faecalis* (55%) and *Streptococcus devriesei* (45%) were the most prevalent species in donkey milk. No other LAB belonging to the most technically important genera was identified. Biochemical and sugar fermenting analyses confirmed the results of 16S rRNA sequencing. Both species identified were sensitive to nearly all antibiotic tested, including vancomycin. Few strains were found to have >99% sequence similarity with *Streptococcus devriesei*, indicating the presence of this LAB in donkey milk. This is the first study to investigate the LAB microbiota present in donkey's milk in IRAN.

**FEMS-1415**

**Food microbiology**

**BIOGEOGRAPHY OF NON-SACCHAROMYCES YEASTS ASSOCIATED WITH SPONTANEOUSLY FERMENTED MUSTS FROM DIFFERENT VINEYARDS AND WINE PRODUCING REGIONS IN GREECE**

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**Background**

Non-*Saccharomyces* (wild) yeasts may confer diverse chemical composition and improved sensory properties to wines. Yet, our knowledge on population structure of these species is limited.

**Objectives**

Species diversity and population structure of 486 wild yeasts associated with spontaneously fermented musts from two geographically separated regions in Greece, Peza and Nemea, were assessed. The efficiency of different fingerprinting methods in resolving yeast populations was evaluated.

**Methods**

Species identification was conducted by PCR-RFLP analysis. For molecular typing the tandem repeat-tRNA (TRtRNA)-PCR and RAPD analysis with primers (GTG)<sub>3</sub>, R<sub>5</sub> or RF<sub>2</sub> were applied. Molecular patterns and vineyard populations were clustered by Principal Coordinates Analysis and UPGMA, respectively. Differences of populations were evaluated by analysis of molecular variance (AMOVA).

**Conclusions**

Ten populations of wild species were identified and further genotyped by TRtRNA-PCR with the primer ISSR-MB (*Lachancea thermotolerans*, *Metschnikowia pulcherrima* and *Hanseniaspora osmophila*), and RAPD with the primer RF<sub>2</sub> (*Candida zemplinina*), (GTG)<sub>3</sub> (*Hanseniaspora uvarum* and *Hyphopichia pseudoburtonii*) or R<sub>5</sub> (*Issatchenkia orientalis*, *Pichia anomala*, *Torulaspora delbrueckii*, and *Hanseniaspora guilliermondii*). Except for *I. orientalis*, the percentage of biodiversity of yeast populations was higher in Nemea than in Peza. No particular grouping of banding patterns according to the sampling point or to the vineyard of origin was observed.

However, AMOVA showed that populations of *C. zemplinina* and *L. thermotolerans* differed significantly between the two regions. Results point to the presence of region specific sub-populations of non-*Saccharomyces* yeasts species that could enhance the Greek wines distinctiveness.

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**FEMS-2547**

**Food microbiology**

## **SELECTION OF INDIGENOUS YEASTS FOR PRODUCTION OF QUALITY TERROIR-DRIVEN GREEK WINES**

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<sup>2</sup>Department of Enology, TEI of Athens, Athens, Greece

### **Background**

According to the microbial *terroir* concept, the use of indigenous yeasts as starters in winemaking may add to the uniqueness of regional wines.

### **Objectives**

Genetically different yeast strains from spontaneously fermented musts from Santorini island, Mantinea plateau and Nemea region were characterized phenotypically in order to select strains with desirable enological properties to be used as starters.

### **Methods**

Strains belonging to *Candida zemplinina*, *Issatchenkia orientalis*, *Hanseniaspora quilliermondii*, *H. opuntiae*, *Lachancea thermotolerans*, *Saccharomyces cerevisiae* and *Torulaspora delbrueckii* were evaluated for tolerance to ethanol, SO<sub>2</sub> and different temperatures, H<sub>2</sub>S and biogenic amines production, maximum population size, foam generation, killer and flocculation phenotype.

### **Conclusions**

Most of *S. cerevisiae* strains tolerated the selective pressures assayed, exhibiting growth at 16% ethanol, 250 ppm SO<sub>2</sub> or 37 °C. Lower tolerance limits were observed for non-*Saccharomyces* yeasts, with strains of *C. zemplinina*, *I. orientalis* and *T. delbrueckii* growing well at concentrations of 14% ethanol or 200 ppm SO<sub>2</sub>. Most strains did not produce histamine, tyramine, putrescine, cadaverine phenylethylamine, tryptamine and isoamylamine, presented low foaming, no-flocculation or killer phenotype and were resistant to killer toxins. Substantial intra- and inter-species differences were observed in H<sub>2</sub>S production. Present results highlight the phenotypic diversity among indigenous yeasts and reveal strains with prominent oenological traits that could be exploited in local wine production.

### **Acknowledgements**

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**FEMS-0864**

**Food microbiology**

## **DEVELOPMENT AND EVALUATION OF YOGHURT FLAVOURED WITH TAMARIND (TAMARINDUS INDICA)**

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### **Background**

Yoghurt is produced by bacterial fermentation of milk sugar (lactose), using *Streptococcus thermophilus* and *Lactobacillus bulgaricus*, producing lactic acid give yoghurt its texture and its characteristic tang. To modify certain properties of yoghurt, the fermented milk could be blended with ingredients fruits (for flavor and color) and sucrose or aspartame (sweetness). Exotic natural or artificial flavors to boost yoghurt like vanilla, orange, pineapple, strawberry, raspberry, cinnamon among others. However, tropical underutilized flavours like tamarind (*Tamarindus indica*) have not been exploited.

### **Objectives**

To develop and evaluate yoghurt flavored with Tamarind chemically, microbiologically and organoleptically.

### **Methods**

Tamarind fruit was pulped to juice and treated in three ways (sweetened with sugar, honey or without sweetener/ Skimmed milk and water are homogenized, pasteurized for 15 mins at 85°C, and cooled to 42 - 44°C. The starter culture inoculated, mixed and incubated for 12 hour fermentation, flavoured with graded levels (0, 2, 4, 6, 8, 10, 20, 30, 40, 50 %.) of tamarind juice and stored for 0 to 21 days with weekly monitoring. The sample was then subjected to proximate, micro-nutrient, microbiological and sensory analysis.

### **Conclusions**

Micro-nutrient content (Mg, Na, K, C and Vitamin C) increased with the addition of the fruit juice. Total solids increased with increase concentration of the sweetened fruit flavour in yoghurt. There was no mould or coliform growth but the total viable count ranged from  $6.3 \times 10^2$  to  $2.34 \times 10^7$  cfu/ml after 21 days. The sugar sweetened samples were most acceptable over the honey and fruit juice without sweetener.



**FEMS-2966**

**Food microbiology**

## **HIGH-THROUGHPUT SEQUENCING REVEALS BIOGENIC AMINE FORMING COMMUNITIES IN A VARIETY OF ARTISANAL CHEESES**

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### **Background**

Biogenic amines are nitrogenous compounds with biological activity produced, primarily, by a variety of lactic acid bacteria. Biogenic amines, such as histamine and tyramine, are commonly found in fermented food products. In cheese, high concentrations can result in adverse health effects for the consumer.

### **Objectives**

The aim of this study was to screen a range of artisanal cheeses (n=10) for the presence of microbial populations capable of producing biogenic amines.

### **Methods**

Segments of the histidine (*hdc*) and tyrosine (*tdc*) decarboxylase genes were amplified using previously published PCR primer pairs. PCR amplicons were then TOPO-cloned and subjected to Sanger sequencing. In addition, the Ion Torrent PGM sequencer was used to provide a novel, complementary, in depth analysis of amine forming communities.

### **Conclusions**

Analysis of both Sanger and Ion PGM sequence data revealed *hdc* and *tdc* positive bacterial populations both within cheeses and across different cheese varieties. *Lactobacillus curvatus*, *Lb. brevis*, *Enterococcus faecium* and *E. faecalis* were identified as the predominant species capable of producing tyramine while *Lb. buchneri*, *Lb. sakei* and *Lactococcus lactis* were among the species found to harbour histaminogenic potential. High-throughput Ion sequencing alone revealed the presence of sub-dominant genera, including, among others, *Staphylococcus saprophyticus* and *Streptococcus thermophilus*. Actual levels of histamine and tyramine in the respective cheeses were quantified by HPLC.



**FEMS-2862**

**Food microbiology**

**BACTERIOLOGICAL QUALITY OF COMMERCIALLY PREPARED FERMENTED OGI (AKAMU) SOLD IN SOME PARTS OF SOUTH EASTERN NIGERIA**

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**Background**

Food poisoning and infection by bacteria are of public health significance to both developing and developed countries.

**Objectives**

This study compared the bacteriological qualities of commercially and laboratory based fermented ogi (akamu) prepared from maize.

**Methods**

Samples of ogi (akamu) prepared from white and yellow variety of maize sold in Uturu and Okigwe were analyzed together with the laboratory prepared ogi for microbial quality using the standard microbiological methods. The analyses showed that both white and yellow variety had total bacterial counts (cfu/g) of  $4.0 \times 10^7$  and  $3.9 \times 10^7$  for the laboratory prepared ogi while the commercial ogi had  $5.2 \times 10^7$  and  $4.9 \times 10^7$ ,  $4.9 \times 10^7$  and  $4.5 \times 10^7$ ,  $5.4 \times 10^7$  and  $5.0 \times 10^7$  for Eke-Okigwe, Up-gate and Nkwo-Achara market respectively. The Staphylococcal counts ranged from  $2.0 \times 10^2$  to  $5.0 \times 10^2$  and  $1.0 \times 10^2$  to  $4.0 \times 10^2$  for the white and yellow variety from the different markets while Staphylococcal growth was not recorded on the laboratory prepared ogi. The laboratory prepared ogi had no Coliform growth while the commercially prepared ogi had counts of  $0.5 \times 10^3$  to  $1.6 \times 10^3$  for white variety and  $0.3 \times 10^3$  to  $1.1 \times 10^3$  for yellow variety respectively. Lactic acid bacterial count of  $3.5 \times 10^6$  and  $3.0 \times 10^6$  was recorded for the laboratory ogi while the commercially prepared ogi ranged from  $3.2 \times 10^6$  to  $4.2 \times 10^6$  (white variety) and  $3.0 \times 10^6$  to  $3.9 \times 10^6$  (yellow).

**Conclusions**

There are chances of contracting food borne diseases from commercially prepared ogi. Therefore, there is the need for sanitary measures in the production of fermented cereals so as to minimize the rate of food borne pathogens during processing and storage.

**FEMS-0560**

**Food microbiology**

## **ISOLATION AND MYCOTOXIGENIC ACTIVITIES OF SOME MYCOFLORA OF WEST AFRICAN CHEESE**

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### **Background**

Cheese(wara) is a local dairy product produced and consumed in many African countries. It is produced locally by the Fulanis who use *Calotropis procera* leaves as coagulating agent for the milk.

### **Objectives**

The isolation and identification of the fungi species present in wara and their ability to produce mycotoxins was carried out to determine the safety level associated with consumption of the cheese.

### **Methods**

Mycotoxin production was determined with the use of the enzyme-linked immunosorbent serological assay (ELISA) technique

### **Conclusions**

Some of the isolated mycoflora were found to be mycotoxigenic producing aflatoxins at various levels.

**FEMS-1195**

**Food microbiology**

**INVITRO PROTEIN DIGESTIBILITY OF FERMENTED BAMBARA NUT  
(VOANDZEA SUBTERRANEAN L. THOUARS) USING DIFFERENT SPECIES OF  
RHIZOPUS**

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**Background**

Bambara nut as legume contains sufficient quantities of carbohydrate and protein high in lysine and methionine. Processing methods, such as soaking, cooking or fermentation can improve the quality of legume protein.

**Objectives**

Therefore, this research considered the effect of fermentation on *in vitro* protein digestibility of bambara nut

**Methods**

The bambara nut was obtained from a local market in Ogbomoso, Nigeria. Different species of *Rhizopus* for the fermentation were obtained from Ladoké Akintola University of Technology, Ogbomoso, and the Institute of Agricultural Research and Training, Ibadan to produce fermented bambara nut flour. The *In vitro* protein digestibility of the flour sample was consequently evaluated.

**Conclusions**

Fermentation process using *Rhizopus* species alone, and with their combinations showed improvement in the nutritive value of the fermented bambara nut in relation to *in-vitro* protein digestibility, and the highest rate of protein digestibility was obtained using *R. oligosporus* alone

**FEMS-2979**

**Food microbiology**

## **SEPARATION OF LACTOBACILLI BY MEANS OF MOLECULAR TOOLS**

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### **Background**

Various Lactobacillus species are responsible for the production of fermented food products. Prior to the use of a certain isolate for industrial purpose, it is advisable to classify it at least on species level. Nevertheless, closely related species are not easy to distinguish but molecular methods might be able to reveal the differences even on subspecies level.

### **Objectives**

The aim of our experiments was to verify the usability of several molecular methods in discrimination of Lactobacillus species and compare the efficiency of these tools.

### **Methods**

DNA of lactobacilli were subjected to investigations by PCR (general bacterial and species-specific primers) and qPCR (including newly developed primers and High Resolution Melting - HRM), RFLP and sequencing in order to compare the efficiency of these methods in separation of species.

### **Conclusions**

The HRM had proved its power as a one-step tool for preliminary classification of lactobacilli. With this method it might be much easier to separate closely related species, though, additional techniques, e.g. traditional PCR (species-specific primers), sequencing and RFLP pattern analysis might be necessary to use in order to confirm the results.



**FEMS-2082**

**Food microbiology**

**GENOMICS OF STREPTOCOCCUS MACEDONICUS: MOVING FROM PATHOGENICITY TO ADAPTATION TO THE DAIRY ENVIRONMENT**

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**Background**

Lactic acid bacteria (LAB) constitute a significant group of microorganisms for food fermentations and for human health. The *Streptococcus bovis*/*Streptococcus equinus* complex (SBSEC) within LAB includes members that have been implicated in human disease, but are also found in foods.

**Objectives**

In this study we compared the three available genome sequences of *S. macedonicus* strains isolated from dairy products. Only one strain has its genome complete sequenced and previous analysis showed diminished pathogenic potential and adaptation to the milk environment. Here we present the *in silico* analysis of these strains, in order to better understand the *S. macedonicus* species.

**Methods**

Chromosomal maps were constructed using DNAPlotter and whole genome sequence alignments were performed by progressiveMAUVE and Webact in order to visualize conserved genomic regions or chromosomal rearrangements. Genomic islands were identified and visualized by IslandViewer, potential bacteriocins were predicted by BAGEL3 and CRISPRs were analyzed by the tools available in the CRISPRcompar web-service.

**Conclusions**

The analysis revealed that *S. macedonicus* strains have lost genes involved in the catabolism of complex plant carbohydrates, in the adhesion to the host's cells and in haemolysis that are present in pathogenic SBSEC. In addition, *S. macedonicus* carries two lactose operons and a proteolytic system characteristic of dairy LAB. Our whole genome analysis of *S. macedonicus* shows adaptation traits to the nutrient-rich milk environment.

**FEMS-1810**

**Food microbiology**

## **INACTIVATION OF BROCHOTHRIX THERMOSPACTA BY ATMOSPHERIC COLD PLASMA.**

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### **Background**

*Brochothrix thermosphacta* is considered one of the predominant food spoilage organisms in meat. Atmospheric cold plasma technology (ACP) is proposed as a potential technology for elimination of microbial contamination in food products.

### **Objectives**

The aim of the study was to investigate the antimicrobial efficacy of dielectric barrier discharge atmospheric cold plasma (DBD ACP) for inactivation of *B. thermosphacta*.

### **Methods**

A large gap DBD ACP system was used to investigate the inactivation of *B. thermosphacta* ( $10^{7-8}$ CFU/ml) in PBS, liquid meat model medium (beef extract broth) and raw lamb chops. Samples were placed inside sealed rigid polypropylene containers and were treated with cold plasma using modified atmosphere as the inducer gas. The effect of ACP critical control parameters, such as treatment time, voltage level, media composition and post treatment temperature storage conditions against both planktonic bacteria and biofilms were evaluated. The surviving bacterial populations were estimated by colony count assay.

### **Conclusions**

ACP showed substantial reductions of bacteria depending on the growth media. ACP treatment (80kV) for 30s completely inactivated the bacterial population in PBS, while 5 min treatment showed 2 Log unit bacterial reduction with the meat model medium. The antimicrobial efficacy of plasma against bacteria on lamb chops showed a reduction around 0.5 log units over a 10 day storage period. The results indicate ACP could be effective for inactivation of *B. thermosphacta* if treated with sufficient treatment periods providing a prolonged shelf life for meats and meat products. Further studies will investigate the effect of extended ACP treatment times for complete inactivation.

**FEMS-1776**

**Food microbiology**

## **LARGE-SCALE GENE DELETION IN LACTOCOCCUS LACTIS USING THE CRE-LOXP RECOMBINATION SYSTEM**

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### **Background**

Minimum genome factories (MGFs) can be defined as recombinant strains whose metabolic pathways have been optimized for special applications. The plasticity of genomes gives us hints that it is probable to streamline physiological pathways through genome rearrangements as strains possess a number of non-essential genes. Lactic acid bacteria (LAB) are low G-C content Gram-positive bacteria and have relatively small genome. *Lactococcus lactis* could be served as the starting platform for value-added metabolites biosynthesis. Construction of a set of chassis cells provides the opportunity to obtain specific strains with desired performance.

### **Objectives**

To delete the large-scale genes in *Lactococcus lactis* and construct the set of chassis cells for further synthetic biology research.

### **Methods**

The Cre-*loxP* recombination system was used to construct a large scale region (19.0 kb) deficient strain N8 $\Delta$ L1. Two *loxP* sites were integrated into the genome at target sites, and the large scale region (L1) was replaced by a *cat*-cassette. The recombination between two *loxP* sites was achieved when the Cre recombinase was expressed by constructed pNZTS-Cre plasmid, and then the L1-null strains were isolated. Despite the loss of 19.0-kb regions, the strain N8 $\Delta$ L1 still exhibited normal growth and similar growth profile was also observed compared to the parental strain.

### **Conclusions**

The Cre-*loxP* recombination system can be used for large-scale genome deletion and generate sequential chromosomal modification mutants of *L. lactis* efficiently. Based on the precision of its deletion, further genome streamlining and advance the regulatory network study about synthetic biology could be easily achieved.

**FEMS-2741**

**Food microbiology**

## **EFFECT OF RESPIRATIVE METABOLISM OF LACTOBACILLUS CASEI ON MODEL SOURDOUGH FERMENTATION**

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### **Background**

*Lactobacillus casei* is a lactic acid bacterium (LAB) used in the production of many fermented food and feed products. Furthermore, this specie comprises strains commercially exploited as probiotic cultures. Thus, novel strategies to improve their adaptation to adverse conditions could enhance their safety, functional and technological properties. Recently, it has been demonstrated that respiratory metabolism results in this species in the expression of a phenotype with enhanced technological and stress response properties (increase in biomass, synthesis of antioxidant enzymes, robustness to stress conditions).

### **Objectives**

The aim of the study was evaluating the impact of respirative/anaerobic metabolism of *L. casei* N87 strain on model sourdough fermentation.

### **Methods**

The strain *L. casei* N87, grown both in anaerobic and respirative conditions, was inoculated as starter in wheat flour in combination with a commercial baker's yeast culture. After 0, 6 and 24 h of fermentation LAB count, pH and Titratable acidity values, free amino acid (FAA), volatile compounds (VOCs) (by SPME-GC-MS), antioxidant activity (by DPPH assay) and albumins/globulins, gliadins and glutenins protein fractions (by SDS-PAGE) were evaluated.

### **Conclusions**

A major increase in biomass for the strain *L. casei* N87 grown in respirative condition was registered after 6h of fermentation. Results obtained by FAA and VOCs evaluation indicated that respirative metabolism of *L. casei* N87 influences the flavour of sourdough. Furthermore, SDS-PAGE highlighted a different wheat protein degradation in anaerobic/respirative cultures. In conclusion, respirative *L. casei* N87 performs well in sourdough definition and a better explanation of its role in sourdough production is considered opportune.



**FEMS-2744**

**Food microbiology**

**MICROBIAL CHARACTERIZATION, TOTAL POLYPHENOLS CONTENT AND ANTIOXIDANT ACTIVITY OF CHENOPODIUMQUINOA AND AMARANTHUS SEEDS FROM CAMPANIA REGION**

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**Background**

*Chenopodium quinoa* and *Amaranthus* spp. are considered pseudocereals characterized by high nutritional and functional values which are associated with the quality and quantity of their proteins, fats and antioxidant compounds. In the recent years, these pseudocereals have attracted the interest of research for their exploitation in the production of gluten-free products and food products with improved nutritional and health benefits.

**Objectives**

To characterize quinoa and amaranth seeds (produced in Campania Region) for microbiological, technological and safety aspects with the aim to use them in the production of “functional foods”

**Methods**

Three samples of *Amaranthus* (accessions A1, A2 and A3) and three samples of *Chenopodium quinoa* seeds (2 Titicaca and 1 Puno varieties) were obtained by experimental fields of Vitulazio (Campania Region). Total mesophilic count, lactic acid bacteria (LAB), yeasts, moulds, *Enterobacteriaceae*, enterococci, total and faecal coliforms were assessed by standard pour-plate technique by using selective media. Total polyphenols content was determined using a method based on Folin-Ciocalteu reagent, while antioxidant activity was carried out spectrophotometrically using DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging.

**Conclusions**

Results evidenced a high LAB concentration in quinoa and amaranth seeds ranged between 2.5 and 7.5 log CFU/g and between 2.8 and 5.0 log CFU/g, respectively. Quinoa seeds showed higher level of polyphenol content compared with amaranth, whereas no significant differences were observed in antioxidant activity, registered in high concentration for both the seeds. In conclusion, the characteristics of these seeds are highly desirable for the production of both gluten-free products and food with high nutritional value.

**FEMS-1835**

**Food microbiology**

**UNDERSTANDING DIVERSITY OF BACTERIAL COMMUNITIES FROM POULTRY MEAT TO IMPROVE FOOD QUALITY AND SAFETY**

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**Background**

Meat products can host variable microbial communities according to seasonal changes and production processes. These may encompass pathogenic or spoilage bacteria which must be controlled to ensure safety and quality of the products.

**Objectives**

Our aim was to describe the microbial community of chicken legs packaged under modified atmosphere.

**Methods**

Whole bacterial populations were collected and stored frozen at -80°C. Their ability to regrow on meat was checked. Bacterial diversity was determined by cultural methods (23 samples) and by 16S rDNA pyrosequencing (10 samples).

**Conclusions**

Total viable counts varied with samples ( $10^3$  -  $10^8$  CFU/g) and plating methods showed that lactic acid bacteria, *Brochothrix thermosphacta*, and *Pseudomonas* spp. were the main bacterial. The characterization of the bacterial diversity by 16S rDNA pyrosequencing confirmed the presence of *B. thermosphacta*, and revealed that *Pseudomonas* was mainly represented by *P. extremaustralis* and *P. cedrina*. As well the main LAB species were *Carnobacterium* and *Shewanella* species. The predominance of *Pseudomonas* was correlated to meat packaging under high oxygen concentration, except when *B. thermosphacta* was dominant, suggesting a competition between these species. No clear cut correlation could be observed between farming practices or meat processing and bacterial communities. However, our results showed similar microbial profiles of samples issued from the same slaughterhouse suggesting the main contamination may occur during this processing. The frozen communities will now be used to evaluate the abiotic and biotic (e.g. the *Brochothrix-Pseudomonas* competition) factors in reproducible challenge tests on meat to finally improve the storage life-time

**FEMS-1577**

**Food microbiology**

## **ANTIFUNGAL ACTIVITY OF AQUEOUS POLYPHENOLIC EXTRACTS FROM CITRUS PEELS**

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### **Background**

Fungal growth is the main cause of fruit decay, and is usually control by the application of synthetic fungicides, however, currently their used have been restricted. Therefore, it is urgent to find alternative antifungal substances. Phenolic compounds are secondary metabolites of plants and naturally present in fruit which have been associated with antimicrobial effect. Citrus peel contains high amount of flavonoids and is the main byproduct of processing industry

### **Objectives**

Then, the aim was to investigate the inhibitory activity of phenolic extracts obtained from citrus peel against fungal fruit spoilage.

### **Methods**

The antifungal activity of extracts from orange and lemon peels were tested *in-vitro* against 6 yeast strains (*Cryptococcus* spp., *Torulaspora* spp, *Aerobasidium pullulans*, *Rhodoturula* spp., *Hanseniaspora uvarum*., *Meyerozyma caribbica*) and 8 molds (*Botrytis cinerea*, *Monilia laxa*, *Alternaria* spp., *Penicillium glabrum*, *Penicillium expansum*, *Penicillium corylophilum*, *Cladosporium uredinicola*, *Cladosporium cladosporioides*), by following the ability to grow in a medium containing different concentrations of extracts.

### **Conclusions**

Overall, the inhibitory effect of orange extracts (OE) against yeast strains was around 90% at concentrations higher than 0.75 g/L, whereas the effect of lemon extracts was more strain dependent and powerful, with values closed to 90% against four strains at 0.15 g/L. On the other hand, although inhibitory effect against molds was lower, OE presented a remarkable activity against *B. cinerea*, *M. laxa* and *Alternaria* spp. and moderated against *Penicillium* and *Cladosporium* strains. In conclusion, these results suggest the possibility of using citrus polyphenolic extracts as a safer alternative to synthetic fungicides to control postharvest decay of fruit.





**FEMS-1827**

**Food microbiology**

**LISTERIA MONOCYTOGENES OF SEQUENCE TYPE 121 HARBOR SPECIFIC ADAPTATIONS SUPPORTING PERSISTENCE IN FOOD PRODUCTION PLANTS.**

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**Background**

The foodborne pathogen *Listeria monocytogenes* is able to survive for months and even years in food production environments. Among a great strain diversity particularly strains belonging to sequence type (ST)121 are persistent in food production plants.

**Objectives**

To elucidate the molecular determinants responsible for persistence of *L. monocytogenes*.

**Methods**

We analysed the genomes of two *L. monocytogenes* ST121 strains, which persisted for up to eight years in food production plants in Ireland and Austria. Additionally we characterized two ST121 strain-specific genetic features: Tn6188 and *lin0464/lin0465*.

**Conclusions**

All ST121 genomes are highly similar and show a tremendously high degree of conservation among prophages and particularly among their plasmids, which are usually variable parts of genetic information in bacteria. This remarkably high level of conservation suggests a strong selective pressure.

In addition, all ST121 strains share adaptations related to persistence in food production environments such as the presence of Tn6188, a transposon responsible for increased tolerance against quaternary ammonium compounds, and the presence of homologues of the *L. innocua* genes *lin0464* and *lin0465*, a transcriptional regulator and a putative pfpl protease. Deletion of *lin0465* resulted in reduced survival under oxidative and alkaline conditions suggesting a role in stress response. Furthermore all ST121 strains reveal a yet undescribed insertion harboring recombination hotspot (RHS) repeat proteins, which are most likely involved in

competition against other bacteria. In conclusion we show that *L. monocytogenes* ST121 strains are highly similar to each other harboring conserved regions which provide fitness adaptations to survival in food production environments.

**FEMS-1845**

**Food microbiology**

**NOVEL VIRULENCE FEATURES OF LISTERIA MONOCYTOGENES ISOLATED FROM FOOD SAMPLES SOLD AT A ROMANIAN BLACK MARKET.**

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**Background**

*Listeria monocytogenes* is a facultative intracellular foodborne pathogen responsible for listeriosis. Investigated neglected exogenous routes of transmission of foodborne pathogens into the EU, we have isolated 15 *L. monocytogenes* strains in food products illegally sold at a Romanian black market.

**Objectives**

The aim of this study was to characterize the subtype and the virulence features of these *L. monocytogenes* strains.

**Methods**

We determined invasion efficiency and intracellular growth in human intestinal epithelial Caco2 and macrophage-like THP1 cells; and analysed the sequence of three main virulence factors: PrfA, internalin A (InlA) and listeriolysin O (LLO).

**Conclusions**

Multilocus sequence typing revealed that these *L. monocytogenes* strains belong to six different sequence types (ST). *In vitro* virulence assays showed a high strain variability regarding the invasion efficiency in Caco2 cells and the intracellular growth rate in both cell types. In parallel we revealed a high diversity in the InlA and LLO amino acid sequences, however strains belonging to the same ST harbour identical sequences. We detected in total 30 different amino acid substitutions, resulting in seven different InlA variants, two of which have not yet been described. All ST121 and ST9 strains, harbouring a premature stop codon resulting in truncated InlA, were unable to invade Caco2 cells. In addition the number of LLO mutations correlated negatively with intracellular growth in both cell types; and all ST155 strains showed no proliferation inside macrophages. In conclusion, we show a high diversity in the amino acid sequence of main virulence factors in *L. monocytogenes* resulting in distinct virulence profiles.



**FEMS-2013**

**Food microbiology**

**CELL-CONTACT DEPENDENT COMPETITION OF *L. MONOCYTOGENES* STRAINS EFFECTS FITNESS AND VIRULENCE.**

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**Background**

Interactions of bacteria living in the same microenvironment influence the behaviour of pathogens like *Listeria monocytogenes*. Therefore inter-strain competition between different *L. monocytogenes* strains could influence fitness and pathogenicity.

**Objectives**

This study investigated the impact of co-culture on growth and *in vitro* virulence of *L. monocytogenes* strains and the effect of cell-contact on the observed growth and virulence competition.

**Methods**

Growth of eight *L. monocytogenes* strains was determined in single and two-strain mixed cultures in tryptic soy broth at 10°C for 10 days. The effect of strain competition (24h, 10°C) on invasion efficiency and intracellular growth was investigated using human intestinal epithelial Caco2 cells. Two selected strains were inoculated singly, mixed or separated by 0.4µm PET-membrane to analyzed whether the observed effects are cell-contact dependent.

**Conclusions**

Significant differences in growth between single and mixed cultures were observed for certain strains e.g. ScottA and 6179 showed reduced growth rates when co-cultivated with other strains. Cell-contact was essential for growth competition.

High virulent strain e.g. PL25 showed either increased or unchanged invasion efficiency when co-cultured with low virulent strains, whereas the invasiveness of low virulent strains like ScottA and 6179 was attenuated. These results suggest a competitive advantage for the strains displaying higher invasion efficiency. In parallel to growth the effect of strain competition on virulence is cell-contact dependent.

In conclusion competition between *L. monocytogenes* strains has a strain-dependent effect on fitness and virulence.



**FEMS-0592**

**Food microbiology**

## **NEW INSIGHTS OF A NATURAL GUM: FOCUSING ON GUT ECOLOGY.**

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### **Background**

Gum odina obtained from bark of *Odina wodier* (Anacardiaceae) is evaluated as prebiotic in this study. The natural gum (polysaccharide) is chemically galactopyranose associated with arabinose side chain.<sup>[1]</sup>

### **Objectives**

The use of prebiotic to fortify gut flora is current aspect of research for the control and prevention of colon cancer as well as boosting of immune system.

### **Methods**

*In vivo* prebiotic potential of gum odina was performed on Swiss albino mice by deliberate challenging of *S.typhi* 62 focusing on immunological parameters<sup>[2]</sup> and *in vitro* studies was investigated by Simulator for Human Intestinal Microbial Ecosystem (SHIME).<sup>[3]</sup>

### **Conclusions**

The non-degradability of gum odina by simulated gastric fluid (SGF) and simulated intestinal Fluid (SIF) attributes to one of the major characteristics of prebiotics i.e. indigestible carbohydrate. Microbial analysis of SHIME studies shows increase in colonization of various probiotic organisms such as *Lactobacillus* sp. and *Bifidobacterium* sp. upon administration of odina. *In vivo* study revealed natural gum selectively stimulates probiotic strains and eliminates enteric pathogens and also boosts immune system by increasing sIgA in gut. Moreover increase in levels of IFN  $\gamma$  signifies additional protection against various pathogen induced primary and secondary infections. This gum is also believed to possess colon carcinoma preventing properties as it liberates short chain fatty acids (SCFA) upon fermentation which we are planning to focus in our next studies.



FEMS-3096

Food microbiology

## USE OF BACTERIAPHAGE-BASED SOLUTION AND ELECTROLYZED OXIDIZING WATER AS ALTERNATIVE SANITIZING SOLUTIONS TO INACTIVATE LISTERIA MONOCYTOGENES ON FOOD CONTACT SURFACES FOR THE SEAFOOD PROCESSING FACILITIES

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### Background

*Listeria monocytogenes* is an important foodborne pathogen that can cause serious public health-related issues worldwide. *L. monocytogenes* could be isolated from food contact surfaces (FCS) from the seafood processing facilities, e.g., slicers, utensils, knives, sieves, conveyors, towels, employer's gloves, which could then link to the occurrence of cross-contamination of seafood products.

### Objectives

Effective sanitizing agents are thus needed to reduce the incidence of *Listeria* contamination on FCS. In this study.

### Methods

Phage-based solution LP-124 (LPS; broad host-range listeriaphage) and electrolyzed oxidizing water (EOW; pH 2.5 and ORP 1150 mV) were evaluated for the ability to inactivate *L. monocytogenes* cells that were artificially contaminated on four different FCSs (plastic cutting board, towel, stainless steel spoon, and sieve) at 4 logCFU/cm<sup>2</sup> on a given FCS.

### Conclusions

Results showed that EOW and LPS led to significantly ( $p < 0.05$ ) decrease of *L. monocytogenes* counts, representing about 2 logCFU/cm<sup>2</sup> on a given FCS, [KV1] compared with the counts recovered from washing with water. *L. monocytogenes* counts were not detected in the previously used EOW solution stored at 4 °C during 7 days after used, while about 2.8 logCFU/ml was observed in used phage-based solution after 7 days of storage. Both EOW and LPS can effectively inactivate *L. monocytogenes* on different food contact surfaces, and can further be used as alternative sanitizing solution for the seafood processing facilities.

**FEMS-0533**

**Food microbiology**

**PREVALENCE OF INTESTINAL PARASITES AMONG FOOD HANDLERS OF SARI, NORTHERN IRAN**

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**Background**

Parasitic infection is highly prevalent throughout the developing countries of the world. Food handlers are potential source of infection of many intestinal parasites and other enteropathogenic infections as well

**Objectives**

The aim of this study was to determine the prevalence of intestinal parasites carriers among food handlers attending the public healthcenter laboratory in Sari, northern Iran for annual check-up

**Methods**

The study was performed from August 2011 through February 2012. Stool samples were collected from 1041 male and female food handlers of different Jobs aged between 18 to 63 years and were examined following standard procedures. Sociodemographic, environmental and behavioral data of the food handlers were recorded in a separate questionnaire. Intestinal parasites were found in 161(15.5%) of the studied samples. Seven species of protozoan orhelminth infections were detected. Most of the participants were infected with Giardia lamblia (53.9%) followed by Blastocystis hominis (18%), Entamoeba coli (15.5%), Entamoeba histolytica/dispar (5.5%), Cryptosporidium sp. (3.1%), Iodamoeba butschlii (3.1%) and Hymenolepis nana (1.9%) as an only helminth infection.

**Conclusions**

The finding emphasized that food handlers with different pathogenic organisms may predispose significant risk on the consumers. Routine screening and treatment of food handlers is a proper tool in preventing the food-borne infections.

**FEMS-0972**

**Food microbiology**

## **IDENTIFICATION AND EVALUATION OF ANTIBIOTIC RESISTANCE OF PROBIOTIC STRAINS OF BIFIDOBACTERIA IN BELARUS**

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### **Background**

Accurate identification and assessment of antibiotic resistance of bifidobacteria are essential to secure quality and safety of probiotics and food products, containing these microorganisms.

### **Objectives**

The objective of the work was identification and evaluation of antibiotic resistance of 25 probiotic strains of bifidobacteria, isolated from feces of healthy adults, therapeutical preparations and dairy products, marketed in Belarus.

### **Methods**

Sequencing of 16S rDNA and transaldolase gene, MALDI-TOF MS protein profiling, biochemical testing, analysis of fatty acid methyl esters (FAMES) were used for bifidobacteria identification. PCR-analysis, disc-diffusion and broth micro-dilution methods were applied for determination of antibiotic resistance of bifidobacteria.

### **Conclusions**

Results of molecular-genetic identification of bifidobacteria indicated that 7 strains belong to species *Bifidobacterium animalis* subsp. *lactis*, 7 strains – *Bifidobacterium longum*, 5 strains – *Bifidobacterium bifidum*, 5 strains – *Bifidobacterium adolescentis*, 1 strain – *Bifidobacterium breve*. MALDI-TOF MS analysis confirmed taxonomic affiliation of tested strains and proved to be a rapid and reproducible technique for bifidobacteria identification. Protein biomarkers suitable for species and strain discrimination were defined. Both biochemical testing, based on evaluation of enzymatic activity and carbohydrates fermentation, and FAMES analysis did not provide accurate species identification of bifidobacteria, but were useful for strain differentiation and characterization. Tested strains of bifidobacteria were susceptible to chloramphenicol (100%), penicillin G (96%), amoxicillin (96%), ampicillin (88%), streptomycin (80%), vancomycin (80%), gentamycin (76%), resistant to erythromycin (40%), clindamycin (40%), tetracycline (52%), and harbor genes *tet(W)* (36%), *tet(M)* (16%), *erm(X)* (32%), *erm(B)* (8%). Gene *tet(W)* was detected in all *B. animalis* subsp. *lactis* strains, gene *erm(X)* – all *B. longum* strains.

**FEMS-2227**

**Food microbiology**

**INFLUENCE OF TEMPERATURE AND INOCULUM SIZE ON SPORES GERMINATION PERCENT OF CLOSTRIDIUM ALGIDICARNIS AND CLOSTRIDIUM ESTERTHETICUM**

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**Background**

'Blown pack' spoilage causes high economic losses for Brazilian meat industry.

**Objectives**

This research aimed to determine the influence of temperature and inoculum size on spore germination percent of *C.algidicarnis*, isolated from spoiled vacuum meat and *C.estertheticum* DSM8809, both recognized for their package blowing ability.

**Methods**

A central composite design - 2 factors x five levels - was applied: storage temperature (-2,3,7,11, 15°C) and inoculum size (10<sup>1</sup>, 10<sup>2</sup>, 10<sup>3</sup>, 10<sup>4</sup> and 10<sup>5</sup> spores/mL, previously prepared). Experiments were conducted using RCM media, in anaerobic conditions. Counts were performed daily, during 30 days, using NMP method. Spores germination percent (SGP) for each day was calculated as: SGP (%) = (count of germinated cells/initial spore inoculum) \* 100).

**Conclusions**

In all assays, for both microorganisms, germination was observed. *C.estertheticum* germinated faster than *C.algidicarnis* at lower temperatures: at 3°C/10<sup>2</sup> spores of inoculum, after 1 day all spores, initially inoculated, were germinated and for *C.algidicarnis*, 100% germination was reached after 4 days. In addition, at 11°C/10<sup>2</sup> sp/mL, germination of *C.estertheticum* was slower than *C.algidicarnis*, achieving 100% after 11 days. For inoculum of 10<sup>3</sup> spores/mL, *C.algidicarnis* reached high germination percent quickly at 15°C (52.4% after 2 days) while *C.estertheticum*, at 7°C (39% at 2 days). Increasing initial inoculum size from 10<sup>2</sup> to 10<sup>4</sup> spores/mL/3°C, increased the time for total germination from 4 to 10 days for *C.algidicarnis* and from 1 to 15 days for *C.estertheticum*. These results clearly show that tested temperatures were not enough to inhibit spores germination of both microorganisms, even if initial population is 10<sup>1</sup> spores/mL, emphasizing the need to avoid the vacuum packed red meat clostridial spore contamination before packaging.

**FEMS-1066**

**Food microbiology**

**THE EFFECTS OF ANTIBIOTICS AND DIFFERENT HOSTILE CONDITIONS ON SURVIVAL AND ADHESION OF PROBIOTIC LACTIC ACID BACTERIA IN VITRO IN MODEL OF HUMAN GASTROINTESTINAL TRACT**

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**Background**

The probiotic microorganisms must be able to survive during passing through the hostile conditions of the gastrointestinal tract and have an ability to adhere to the intestinal epithelial cells. Nowadays there are many physical and chemical factors affecting these abilities of probiotics.

**Objectives**

In this work was studied the effects of electromagnetic irradiation at the frequencies 51.8 and 53 GHz (1h) and antibiotic ceftazidime (20 µM) on survival of *Lactobacillus acidophilus* VKMB-1660 *in vitro* in model of gastrointestinal tract.

**Methods**

Bacteria developed in artificial gastric juice (treatment time 20 min) with pepsin (0.3%) and different pHs (2,0, 3,0, 4,0, 5,0) and artificial intestine juice (treatment time 1 h) with bile salts (0.45%) and pancreatin (0.1%) at pH 8.0. Moreover it was studied the effects of antibiotics and different pHs (5.5, 6.5 and 8.0) on adhesiveness of bacteria.

**Conclusions**

The results obtained shown that the colony forming unit number significantly decreased, in artificial gastric juice than in intestine juice in comparison with untreated control samples. The irradiation at both frequencies and ceftazidime significantly decreased the viability of bacterial samples in both gastric and intestine juices. Moreover, ceftazidime suppressed the adhesion ability of bacteria at all pHs used in comparison with untreated samples and adhesiveness was much weaker at pHs 5.5 and 8.0 than at pH 6.5.

Thus, lactic acid bacteria *L. acidophilus* can survive in viable state in the gastrointestinal tract even after irradiation and treatment with antibiotics, but antibiotics can significantly decrease an adhesion of lactic acid bacteria to intestine mucosa.



**FEMS-2524**

**Food microbiology**

## **HYGIENE BARRIERS IN FOOD PROCESSING FACILITIES MAY SERVE AS “TROJAN HORSES” FOR *L. MONOCYTOGENES***

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### **Background**

**Generally, hygiene sluices are efficient barriers against the transmission of spoilage and foodborne pathogen bacteria.**

### **Objectives**

The aim of the study was to determine the efficiency of hygiene sluices on the reduction of hygiene indicator bacteria and zoonotic agents in food processing companies

### **Methods**

We included five food processing facilities located in Upper- and Lower Austria. Samples were taken before and after sanitation comprising personal fingerprints, swab samples from shoes and the hygiene lock, and water residues and investigated according to microbiological standard methods. *L. monocytogenes* were subtyped by Serogroup PCR and pulsed-field gel electrophoresis (PFGE).

### **Conclusions**

One shoe sample each was found positive for *E. coli* (*rfbE* positive) and *Salmonella* spp. after sanitation in slaughterhouse B and RTE-food producer C, respectively.

Coliform bacteria and *L. monocytogenes* on shoes could not be reduced after passing the hygiene locks. In detail, 29% of the shoe samples were detected *L. monocytogenes* positive before and after the foot bath. Most of *L. monocytogenes* isolates were found on shoes of two slaughterhouses and one RTE-food company working staff. Subtyping revealed that the slaughterhouse A harbored *L. monocytogenes* serogroup 1/2a, 3a and 4b, 4d, 4e (n=21) resulting in five PFGE pulsotypes. In slaughterhouse B *L. monocytogenes* serogroup 1/2a, 3a, 1/2c, 3c and 4b, 4d, 4e (n=11) was present, representing five PFGE pulsotypes. In one RTE-food producing company genetic lineage II (1/2a, 3a; 1/2c, 3c; n=10) were over represented, including persistent strains. Hygiene locks, if not efficiently working, could be a reservoir for human pathogen bacteria and widely distributed *L. monocytogenes* clones.

**FEMS-2533**

**Food microbiology**

## **DIVERSITY AND ANTI-LISTERIAL POTENTIAL OF BACTERIAL FLORA ON HARD-CHEESE RINDS**

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### **Background**

Vorarlberger Mountain cheese Protected Designation of Origin (PDO) owes its special characteristics from the raw milk of extensively farmed cows grazing wild herbs on alpine pastures during the summer months.

### **Objectives**

The aim of the study was to determine the diversity of bacterial flora on hard-cheese rinds isolated from artisan Vorarlberger Mountain cheese. A further goal was to test their antilisterial capability.

### **Methods**

The bacterial rind flora of seven cheese samples, including the variety of three ripening cellars (A, B, C) and two different ripening stadiums, were characterized. Furthermore, five cheeses were investigated during a six month period, to determine the variations in bacterial groups on cheese surfaces due to failures or instabilities in cheese-curing. Bacterial cheese flora isolates were enumerated and confirmed applying standard agar isolation methods and 16S rRNA sequencing. The antilisterial potential of 56 cheese rind bacteria was tested in an agar-overlay model against *Listeria* spp. Additionally, cheese rind bacteria with antilisterial potential and a brine-adapted *L. monocytogenes* isolate were introduced in a challenge experiment on cheese during ripening.

### **Conclusions**

The majority of isolates comprised following genera and species: *Brevibacterium* spp. (30.9%), *Staphylococcus equorum* (20.9%), *Corynebacterium casei/variabilis* (11.4%) and *Brachybacterium alimentarium* (10.9%). Antilisterial potential in the agar model was observed from 30.4 % of test strains. In the challenge experiment higher *L. monocytogenes* spike levels ( $10^4$  cfu) were positive after enumeration and enrichment until the end of smearing at day 14. Applying a brine with higher salt concentration (>20%) resulted in higher growth rates during ripening (average  $3.98 \times 10^3$  cfu/g).





**FEMS-1582**

**Food microbiology**

## **MICROBIAL QUALITY OF EDIBLE INSECTS**

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### **Background**

Current animal protein sources will not be sufficient for the growing population. Therefore, alternative solutions need to be found. Edible insects are considered as a sustainable and nutritionally equivalent alternative to meat. Nowadays, there is a lack of profound insight into the microbiological quality of edible insects.

### **Objectives**

The purpose of this research was to investigate the microflora of edible insects in a quantitative and a qualitative way, in particular of the yellow mealworm (*Tenebrio molitor*) and the grasshopper (*Locusta migratoria migratorioides*).

### **Methods**

Living insects for human consumption were purchased from an eco-shop (Belgium). Both insect species were subjected to culture-dependent analyses (classical microbial counts) to determine the microbial load (total aerobic count (TAC), *Enterobacteriaceae*, lactic acid bacteria (LAB), yeasts and moulds (Y&M) and aerobic bacterial spores). In addition, culture-independent analyses (454 pyrosequencing) were performed to gain insight into the bacterial community composition.

### **Conclusions**

Average microbial counts (3 batches, log cfu/g) in mealworms were 8,0 TAC, 7,3 *Enterobacteriaceae*, 7,4 LAB and 5,4 Y&M. Lower counts were observed for sporeforming bacteria ranging between <1,0-3,5 log cfu/g. Grasshoppers showed average counts (2 batches, log cfu/g) of 7,2 TAC, 5,6 *Enterobacteriaceae*, 7,0 LAB, 4,2 Y&M and 4,2 bacterial spores. The culture-independent analyses demonstrated a high bacterial diversity in mealworms and grasshoppers. Most bacteria belonged to the *Enterobacteriaceae* and LAB, however, the dominant bacterial species differed between both insect species. Hence, raw edible insects contain a high microbial load and diversity, including spoilage organisms and potential pathogens, a decontamination step before consumption is required.



**FEMS-1814**

**Food microbiology**

**A TEN-YEAR SPOT-SAMPLING (2005-2014) OF FISH, WATER AND SURFACES  
ALONG THE PRODUCTION CHAIN IN THE NORWEGIAN PELAGIC FISH  
SECTOR.**

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**Background**

Microorganisms are of great importance in post-harvest spoilage of marine resources. Both quality-reducing and potential pathogenic bacteria may reduce the first-hand value, the export potential, and the reputation of the seafood industry.

**Objectives**

To examine microbiological conditions of the commercially most important pelagic fish species harvested in Norway, with respect to quality, hygiene and food safety.

**Methods**

Microbial fish quality was evaluated by heterotrophic plate counts (HPC) and counts of H<sub>2</sub>S-producing bacteria, whereas hygienic- and safety conditions were assessed by analysing for faecal indicator organisms, e.g. presumptive *E. coli* and enterococci, and the pathogens *Listeria monocytogenes*, *Salmonella* and staphylococci. Examined contact points in the production environment at vessels and factories, included surface samples of equipment and water samples associated with fish catch, -storage, and -production. Contact points were examined for HPC, Enterobacteriaceae, presumptive *E.coli*, enterococci, *Salmonella*, and *L. monocytogenes*. For evaluation, an assessment scheme with recommended microbiological limits suitable for fresh pelagic fish products are proposed based on contemporary EU- and Norwegian guidelines.

**Conclusions**

Quality-, hygienic- and food safety conditions were according to the recommended assessment scheme in all samples from 23 samplings. However, 18 samplings had samples that were not optimal and contamination of fish, the pump nozzle, sift box, sorting chambers, storage tanks, landing tanks and conveyor belts were most often recurring. Two samplings revealed that contamination early in the production chain followed the fish throughout the process, thus an increased focus on clean equipment during capture and storage is highly suggested.

**FEMS-2872**

**Food microbiology**

**YEAST BIOFILM DEVELOPMENT AND RHEOLOGICAL PROPERTIES  
DEVELOPMENT ON TURBULENT FLOW REGIMES.**

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**Background**

In food processing lines or in complex equipment, microorganisms are exposed to varying hydrodynamic conditions caused by the flow of liquid food, and biofilms grown under a wide distribution of hydrodynamic strengths.

**Objectives**

The aim of the present work was to investigate the in situ thickness, architecture, and rheological properties of yeast biofilms growing on stainless steel under turbulent flow.

**Methods**

The yeast species used (*Rhodototula mucilaginosa*, *Candida krusei*, *Candida kefir* and *Candida tropicalis*) were isolated from an apple juice industry. Biofilm formation in turbulent flow were performed in a Rotating Disk System (RDS) already described (Brugnoni et al., 2011). Viscoelastic properties of biofilms were determined by small deformation dynamic oscillatory measurements in a Paar Physica rheometer MCR301 (Anton Paar GmbH, Graz, Austria), using parallel plates.

**Conclusions**

Results show yeasts biofilms formed on stainless steel at Reynolds (Re) numbers ranging from 294,000 to  $1.2 \times 10^6$ . These growth phases transform adherent blastospores to well-defined cellular communities. Biofilm formation increases with Re and time. Flow conditions impacted biofilm composition, with a predominance of *C. krusei*. Under turbulent flow in biofilm thickness increased  $>100 \mu\text{m}$  and cell morphology was governed by hyphal structures and rounded cells. Biofilms resulted viscoelastic materials with a solid-like behavior. Rheological values were not significantly affected by flow conditions or growth time. At large deformations their weak structure collapsed beyond a critical strain.

Brugnoni LI, Cubitto MA, Lozano JE. 2011b. Role of shear stress on biofilm formation of *Candida krusei* in a Rotating Disk System. *J. Food Process Eng.* 102:266–271.



**FEMS-1819**

**Food microbiology**

**ROLE OF THE COLD SHOCK DOMAIN FAMILY PROTEINS (CSPS) IN NISIN AND BENZALKONIUM CHLORIDE STRESS TOLERANCE OF LISTERIA MONOCYTOGENES**

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**Background**

*Listeria monocytogenes* is an important foodborne pathogen that causes rare but serious illness (listeriosis) and high mortality in those with diminished immunity. Proteins of the cold shock domain family (Csps) are global gene expression regulators that promote various stress adaptation responses in bacteria. Nisin and Benzalkonium chloride (BC) are widely used antimicrobials for food preservation and disinfection of food processing environments, respectively.

**Objectives**

To investigate the functional role of Csps in the innate resistance of *L. monocytogenes* to nisin and BC stresses.

**Methods**

*L. monocytogenes* EGDe wild type and *csp* deletion mutant strains were phenotypically compared under nisin and BC stress in growth assays. Impact of nisin and BC stress on gene expression was determined using quantitative reverse transcription PCR (RT-qPCR).

**Conclusions**

In absence of Csp functions the sensitivity of *L. monocytogenes* EGDe to nisin and BC stress exposure was increased. Both growth and survival capacities of the *L. monocytogenes* EGDe strain lacking Csps were significantly diminished compared to the wild type strain when exposed to nisin and BC stress in BHI cultures. In addition the Csp-lacking EGDe strain also showed increased susceptibility to cell envelope-targeting antibiotics. Induction of *csp* genes upon exposure to nisin but not BC was also detected using RT-qPCR. A comparative gene expression analysis revealed an altered expression in genes encoding peptidoglycan-binding proteins in absence of Csp functions. Our results suggest that Csp-dependent gene expression regulation

contributes to optimal intrinsic nisin and BC resistance mechanisms in the foodborne pathogen *L. monocytogenes*.



**FEMS-1822**

**Food microbiology**

**GENOTYPIC AND PHENOTYPIC CHARACTERISTICS OF LISTERIA MONOCYTOGENES STRAINS ISOLATED DURING 2011-2014 FROM DIFFERENT FOOD MATRICES IN SWITZERLAND**

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**Background**

*Listeria monocytogenes* is a food-borne pathogen of public health and food safety concern responsible for highly invasive disease (listeriosis) and high mortality in those with diminished immunity.

**Objectives**

The objective of this study was to characterize *L. monocytogenes* strains recovered from different food matrices in Switzerland between 2011 and 2014 with respect to their genotypic and phenotypic properties.

**Methods**

Genotypes were determined through DNA sequencing (MLST) and PCR (stress survival islet (SSI-1), *qacH* and *bcrABC*) methods. BC resistance (BC<sup>r</sup>) was determined based on minimum inhibitory concentrations on MH agar and biofilm formation by the crystal violet staining method.

**Conclusions**

One hundred and forty two strains isolated from various food products between 2011 and 2014 in Switzerland were examined. The strains comprised serotypes 1/2a (64%), 1/2b (7%), 1/2c (12%), 3c (2%) and 4b (15%). There were 61 MLST sequence types (ST) determined including 24 new ST that had not been previously described. Fifty percent of the strains were SSI-1 PCR positive whereas 31% were negative. Remaining strains either harbored smaller than expected PCR amplicon (15%) or the SSI-1 PCR primers failed (4%) to amplify. BC<sup>r</sup> was detected in 25 (18%) strains including 20 (80%) and 3 (12%) that possessed *qacH* and *bcrABC* BC<sup>r</sup> genetic determinants, respectively. Ninety one percent of the strains were (129/142) were classified as weak, 8% (11/142) as moderate and only 1% (2/142) as strong biofilm formers. Our results indicate a high genetic and phenotypic diversity among *L.*

*monocytogenes* strains that contaminate different types of food products in Switzerland.

**FEMS-1869**

**Food microbiology**

## **MICROBIOLOGICAL QUALITY OF FRESHLY CAUGHT FRESHWATER FISH FROM LAKES IN LATVIA**

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### **Background**

Freshwater fishes are commonly used for fish preparations in Latvia and the hygienic status of a lake from there the freshwater fishes were originated may alter microbiological quality of fresh caught fish.

### **Objectives**

The aim of the present study was to detect the microbiological quality of freshly caught freshwater fish in Latvia.

### **Methods**

Altogether, 36 fishes were collected, among them 25, four and seven were European eel (*Anguilla anguilla*), silver bream (*Blicca bjoerkna*) and European perch (*Perca fluviatilis*), respectively. Samples were collected between September and November, 2014 from fishermen from lakes in Latvia. Surface swabs of a 5x5 cm<sup>2</sup> of freshly caught fish skin were examined according to ISO methods for total bacterial count (TBC), *Enterobacteriaceae*, fecal coliforms, psychrotrophic microorganisms, *Salmonella* spp., *Listeria* spp. and *Yersinia* spp.

### **Conclusions**

Counts of TBC, *Enterobacteriaceae*, fecal coliforms and psychrotrophic microorganisms were found to be significantly lower on eels than on perch and silver bream ( $p>0.05$ ). Eels in Latvia are introduced to lakes in elder or glass eel stage in the frame of eel breeding program and this could influence the contamination rates of eel microflora as they are mostly not a native part of freshwater environment. Observed significant differences ( $p>0.05$ ) between the counts of TBC,

*Enterobacteriaceae*, fecal coliforms and psychrotrophic microorganisms may be explained with the hygienic status of lake of origin of freshwater fish. Absence of *Salmonella* spp., *Listeria* spp. and *Yersinia* spp. in tested samples indicates that they do not share pathogens of public health significance.

**FEMS-2510**

**Food microbiology**

**DISINFECTION SYSTEM WITH UVA-LED FOR HYDROPONIC NUTRIENT SOLUTION**

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**Background:** The stable and safe supply of plant-derived food is endangered by unusual weather, environmental pollution, and shortages of water, fossil fuel and plant biomass. When leaf vegetables are grown in the open field, their quality and productivity tend to vary with the local climate, weather conditions and soil fertility. On the other hand, when plants are grown in the greenhouse, their quality and productivity are generally improved. Thus, the plant factory is a useful system for providing the food supply. When hydroponic nutrient solution in the plant factory is contaminated by the pathogens, the pathogens will propagate and spread, quickly. And the importance of the disinfection system for hydroponic nutrient solution is pointed out in recent years.

**Objectives:** The aim of this study was to develop the disinfection system with UVA-LED and estimate the ability of disinfection of nutrient solution.

**Methods:** We developed a circulating disinfection device including a cylindrical disinfection tank of 1L volume. *Escherichia coli* strain (ATCC25922) was used as model microorganism for this study. Inactivation ability was determined by a colony-forming assay.

**Conclusions:** The disinfection system with UVA-LED was able to sterilize the hydroponic nutrient solution in the plant factory. The equation which indicated the relation between log survival ration, fluence of UVA, and energy consumption was lead by the experiments. The disinfection system with UVA-LED was excellent in not using a chemical substance and low damage to crops and hydroponic nutrient solution.

**FEMS-2679**

**Food microbiology**

## **THE CHANGING BACTERIAL POPULATION OF FRESH-CUT ICEBERG LETTUCE DURING STORAGE**

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### **Background**

Minimally processed (fresh-cut) iceberg lettuce is prone to spoilage after being harvested and processed. As the produce is not sterile, bacterial contaminants may proliferate and add to the organoleptic changes.

### **Objectives**

The objective of this study is to describe changes in the bacterial composition of modified atmospheric (MA) packed fresh-cut produce during the storage period at refrigerated temperature.

### **Methods**

Lettuce samples were taken daily during the storage period for microbiological analysis. Plate culturing was used to analyze changes in the total viable count. Application of pyrosequencing of 16S rRNA gene amplicons was used to monitor the changes in the bacterial population. Dedicated quantitative PCR (qPCR) protocols are developed to monitor the dynamics of specific bacterial entities covering *Leuconostoc*, *Lactococcus*, and *Pseudomonas*.

### **Conclusions**

The mesophilic aerobic bacterial counts increased within 8 days from about  $10^5$  to  $10^8$  colony forming units per gram of lettuce. Pyrosequencing showed at the genus level, *Pseudomonas*, *Leuconostoc*, and *Lactococcus*, being most dominant directly after packaging while *Leuconostoc* becomes most dominant while storage time is elapsing. Application of these qPCR protocols confirmed the typical change of the bacterial population of MA packed fresh-cut iceberg lettuce towards high dominant numbers of *Leuconostoc* species at day 8 after packaging. In several experiments, *Leuconostoc* spp. was found to dominate the bacterial composition at the later time points during storage, suggesting a role for *Leuconostoc*-derived metabolites in lettuce quality attributes.

**FEMS-2070**

**Food microbiology**

**ASSOCIATION BETWEEN VIRULENCE PROFILE, SEQUENCE TYPE AND  
SEROTYPE OF SALMONELLA ENTERICA STRAINS ISOLATED FROM  
FOODSTUFFS IN MICHOACÁN, MEXICO**

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**Background**

The products of the genes *invA*, *rmbA*, *ssaQ*, *sopB*, *sopE*, and *sip4-F* are involved in the invasion and infection mechanisms of *Salmonella enterica*. Virulotyping is a new scheme of genotyping based on the detection of these virulence associated genes (Virulence profile or VP) that has been proven to be an efficient tool for identification and clustering of strains/clones of *S. enterica*.

**Objectives**

Evaluate the association between VP with Sequence Type (ST), serotype, and the geographic region of provenance of *Salmonella enterica* strains.

**Methods**

Ninety strains of *S. enterica* isolated from meat and dairy products collected in the State of Michoacán, Mexico with known ST, serotype and provenance were analyzed. PCR products of the genes *invA*, *rmbA*, *ssaQ*, *sopB*, *sopE* and *sip4-F* were included in the study.

**Conclusions**

Eleven different VPs were observed and named alphabetically as A through K. VP-A contained all of the virulence genes analyzed and is widely distributed throughout the state, followed by VP-B and C with the largest number of virulence-associated genes, except for genes *invA* and *sopE*, respectively. The highest diversity of VP was observed in the port of Lázaro Cárdenas, and in the regions of Morelia and Apatzingán. VP was unrelated to ST and serotype of tested strains. Because in all cases at least three virulence-associated genes were found, our results show that the studied strains of *S. enterica* have a high pathogenic potential, which is relevant for

public health given the presence of these strains in foodstuffs of daily consumption in the state of Michoacán.



**FEMS-0987**

**Food microbiology**

## **POTENTIAL USE OF ESSENTIAL OILS FOR SURFACE DISINFECTION**

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### **Background**

Bacteria can attach to different surfaces and form biofilm causing hygiene problems. Microbes in biofilms are more resistant to antimicrobials than planktonic cells. The organic food industry can use only a limited number of disinfectants. Customers prefer “green technologies” in food production and processing thus there is a continuous need for the use of new natural compounds. Essential oils (EOs) have antimicrobial effect, are easy to produce and eco-friendly. Essential oils are hydrophobic and make the membrane more permeable and more hydrophobic on low pH.

### **Objectives**

In the present study we have investigated cinnamon, marjoram and thyme EOs (on the basis of our pre-study) against bacterial biofilm formation on different surfaces.

### **Methods**

MIC/MBC values of the EOs were determined against food spoilage bacteria: *E. coli*, *P. putida* and pathogens: *L. monocytogenes*, *S. aureus*, MRSA, *B. cereus* and *B. subtilis*. Disinfection time depending on pH and EO concentration was established. Biofilm elimination capacity of the EOs (24 and 168 hours old) from stainless steel and polypropylene surfaces (frequently used in food industry) was tested.

### **Conclusions**

Biofilm formation on plastic was stronger, than on stainless steel probably due to the roughness of surfaces. All investigated EOs had good biofilm removing effects. Best result was achieved with marjoram. In most cases bacterial reduction was 99.99%. EOs had also antibacterial effect on the tested isolates of MRSA and *L. monocytogenes*.

**FEMS-1594**

**Food microbiology**

**EFFECT OF DRYING TREATMENT WITH POTASSIUM CARBONATE (K<sub>2</sub>CO<sub>3</sub>) ON SAFETY QUALITY OF DRIED FIG (FICUS CARICA L.), AS AN ALTERNATIVE TO TRADITIONAL PROCESS.**

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**Background**

Sun drying process involves microbial risks such as fungal spoilage which could also produce mycotoxin.

**Objectives**

Therefore, the aim of this work was to study the effect of drying treatment with potassium carbonate (K<sub>2</sub>CO<sub>3</sub>) combined with osmotic solution on safety quality of fig from 'Calabacita' cultivar.

**Methods**

Two treatments of drying were used, firstly figs were dipped into K<sub>2</sub>CO<sub>3</sub> solution (10%) for 30 min, then in a sugar solution (50%) for 24h and finally drying at 65°C in a drier (TP). A second batch, with figs traditionally sun drying, was used as control (ND). Both batches of dried fig were packed in polyethylene punnets with a macroperforated film and stored at 20 °C for 90 days. Moisture loss was monitored during treatment and storage. Moreover, mold counts and mycotoxin presence were study during storage. Finally, molds were isolated and identified by sequence of the ITS region.

**Conclusions**

The moisture level in TP was 22% after two days of treatment, whereas similar value was obtained in ND after 12 days. Then, the used of potassium carbonate combined with sugar solution reduced significantly the time of drying. During storage, mold reached counts of 1.82 and 2.22 log CFU/g for TP and SD, respectively, at 30 days. *Cladosporium cladosporioides* was the main specie followed by *Aspergillus fumigates* in both batches, although they were found in greater number in ND. No mycotoxins were detected in both batches, however, the higher level of mold found in control batch, as well as longer drying process could suppose greater risk of mycotoxin production.



**FEMS-1389**

**Food microbiology**

**PROFICIENCY TESTS FOR CAMPYLOBACTER ORGANIZED BY THE NATIONAL  
REFERENCE LABORATORY FOR FOOD OF ANIMAL ORIGIN IN POLAND  
DURING 2008-2014**

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**Background**

In accordance with the EU regulation No. 882/2004 National Reference Laboratories (NRL) are responsible for organization of proficiency tests (PT) for official food control laboratories. In Poland, NRL for *Campylobacter* was nominated at the National Veterinary Research Institute in Pulawy.

**Objectives**

The aim of the study was to evaluate the proficiency of laboratories by their participation in PTs towards determination of presence and number of *Campylobacter* in samples of food of animal origin.

**Methods**

The PTs were organized between 2008–2014 according to ISO/IEC 17043:2001, ISO/TS 22117:2010, and ISO 13528:2005 standards. In each year, two rounds of PT, i.e. one for detection and one for enumeration of *Campylobacter* in fresh poultry or pork meat, were performed. The samples were contaminated with the target *C. jejuni* or *C. coli* microorganisms at the levels between  $10^2$  -  $10^3$  cfu for 10 g or 25 g (detection) and  $<10^0$  –  $10^5$  cfu/g (enumeration). The samples were sent to the laboratories by the courier post in a cooled conditions which were obliged to begin the analyses within 24 h (detection) or immediately upon receiving the samples (enumeration). The number of official laboratories participated in each PT round varied from 7 (enumeration; year 2010) to 20 (detection; year 2012).

**Conclusions**

Most of the laboratories obtained satisfactory results (70 – 100% participants depending on the PT round) which indicated that food safety is under appropriate supervision. The PTs were the effective tool used by NRL to supervise the official food control laboratories in Poland.

**FEMS-1704**

**Food microbiology**

**A NEW MULTIPLEX REAL-TIME PCR METHOD FOR DETECTION OF  
SALMONELLA SPP., LISTERIA MONOCYTOGENES AND VEROTOXIGENIC  
ESCHERICHIA COLI IN CARCASSES OF SLAUGHTERED ANIMALS**

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**Background**

*Salmonella* spp., *Listeria monocytogenes* and verotoxigenic *Escherichia coli* (VTEC) are among the most dangerous food-borne bacterial pathogens. Therefore, the availability of rapid and specific methods, especially to simultaneous detection of these pathogens, is needed.

**Objectives**

The objective of the study was to develop and evaluate a new TaqMan multiplex real-time PCR method for detection of *Salmonella* spp., *L. monocytogenes* and VTEC in slaughtered animals.

**Methods**

The method includes the enrichment step in Tryptone Soya Broth, DNA extraction and two real-time PCR reactions, one for detection of *invA* and *hly* genes of *Salmonella* spp. and *L. monocytogenes*, respectively, and another for detection of *vtx1*, *vtx2* and *eae* of VTEC. The results of validation showed a relative sensitivity, specificity and accuracy of 100% compared to the reference methods. The limit of detection (LOD) was established for 1 cfu per swab for *Salmonella* spp. and *L. monocytogenes* and 2 cfu for VTEC. The method developed was used to analyze 145 swabs collected at the slaughterhouse level from cattle, pigs and poultry carcasses. Among the 75 samples recovered from cattle 24 were positive for VTEC and two for *Salmonella* spp., whereas swabs from pigs (n=45), 3 and 2 samples, were positive for these pathogens, respectively. None of microorganisms tested were detected in 25 samples from poultry.

**Conclusions**

The method developed allows for rapid and specific detection of three food-borne pathogens in carcasses of slaughtered animals.



**FEMS-1426**

**Food microbiology**

**PROPHAGE-ENCODED STAPHYLOCOCCAL ENTEROTOXIN A: REGULATION OF PRODUCTION IN DIFFERENT STAPHYLOCOCCUS AUREUS STRAINS**

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**Background**

Staphylococcal Food Poisoning (SFP) is one of the most common food-borne intoxication diseases, caused by enterotoxins produced by *Staphylococcus aureus*. 21 staphylococcal enterotoxins (SEs) or enterotoxin-like proteins (SEIs) have been recognized until now with enterotoxin A (SEA) being most frequently implicated in SFP outbreaks. SEA is encoded by the *sea* gene, located on the genome of *Siphoviridae* bacteriophages and unlike most other enterotoxins is not regulated by the accessory gene regulator (*agr*). Previous studies have shown that the regulation of the *sea* gene in the different SEA-producing strains is partially linked to the lifecycle of the prophages carrying the gene. Differences have been observed on the levels of toxin produced among the strains, and are hypothesized to originate from the *Siphoviridae* phages.

**Objectives**

With this study, we aim to get a better insight on the regulation of the *sea* gene by understanding the level on which the phage life-cycle affects SEA production.

**Methods**

We study five *S. aureus* strains under optimal and chemically induced conditions and monitor the replication of the phage in connection with *sea* gene copies and mRNA transcript levels. Furthermore, we follow the extracellular toxin levels and estimate phage titers.

**Conclusions**

The data obtained have showed a distinct correlation between replicating phage copy levels and *sea* gene copy levels in both growth conditions, linking phage induction with the increase observed on SEA levels. The significance of these findings lays in the realization that any treatment potentially inducing replication of the *sea*-carrying prophages, will enhance the risk for SFP.

**FEMS-1959**

**Food microbiology**

## **LISTERIA MONOCYTOGENES CONTAMINATION IN COLD-SMOKED FISHERY PRODUCTS SOLD IN AUSTRIA**

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### **Background**

Smoked salmon as a ready-to-eat (RTE) food can be typically contaminated with *Listeria monocytogenes*, albeit in low numbers. The legal safety limits for RTE are set in the European Commission Regulation No. 2073/2005 and are particularly exceeded by RTE fishery products and cheeses.

### **Objectives**

The aim of the present study was to investigate the *L. monocytogenes* contamination rates in cold-smoked fishery products in Austria over a one-year period (2013-2014).

### **Methods**

A total of 250 RTE samples were collected in the region of Vienna and analysed at the end of shelf-life. The influence of physico-chemical properties (aw, pH and phenolic content) on the prevalence was also assessed. Isolates were characterized by multiplex PCR serogrouping and rep-PCR. The biofilm forming ability of *L. monocytogenes* isolates was assessed by the crystal violet method and a modified fluorescence microscopy-based technique.

### **Conclusions**

Among the tested fishery products 23% were contaminated with *L. monocytogenes* which correlates with international literature data. Enumeration results ranged from 1 to 760 CFU/g. Serogrouping achieved that 87% of isolates have been classified to 1/2a, 3a serotype and others mainly belonged to groups 4b, 4d, 4e and 1/2b, 3b, 7. Biofilm forming ability could be determined for some isolates when compared with *L. monocytogenes* reference strains representing different serotypes and sources. The physico-chemical data revealed a strong variability within the smoked fishery product category. These discrepancies show the need for factory specific analysis of *L. monocytogenes* growth potential in smoked fishery products in order not to misinterpret the risk-categorization of such products.





**FEMS-0709**

**Fungal bacterial interactions**

**ACTINOMYCETES AS BIOCONTROL AGENTS IN GRAPEVINE WOOD DISEASES: A GOOD TENANT IN THE ROOT?**

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**Background**

One of the most important problems in wine production regions is the premature death of young grapevines. It is due mainly by the infection of fungus that produces wood diseases, like Petri disease and black-foot disease. There are two principal ways to infection. One is the 'industrial process' in order to produce the commercial grafted plants. Another possibility is associated to 'natural processes' when the infection is produced on the field mainly by the pruning wounds or by natural infection through the root system. In fact, most of pathogens associated to these diseases are able to infect the grapevine through the root system.

**Objectives**

The main objective of this study is the use of actinomycetes isolated from the rhizosphere of young grapevines as biocontrol agents (BCAs) in order to reduce or avoid the infection through the root system by pathogenic fungus.

**Methods**

Several actinomycetes were isolated from rhizosphere of young plants of *Vitis vinifera*. These were unequivocally identified by molecular methods. Their capability as biocontrol agent were bioassayed *in vitro* against several pathogenic fungus. Those showing the best capabilities were selected for 'field studies' on grafted plants in order to know the real behaviour as BCAs.

**Conclusions**

Several actinomycetes have shown a good capability to reduce the infection rate through the root system of several fungal pathogens causing decline of young grapevines. They could be a good alternative in order to avoid chemical products in the establishment of new vineyards.

## FEMS-3002

### Fungal bacterial interactions

#### EFFECT OF SOME LIGNICOLOUS EDIBLE AND MEDICINAL FUNGAL SPECIES AGAINST VIRAL (BACTERIOPHAGE) AND BACTERIAL DNA

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#### Background

Nowadays, when high resistance against common antibiotics is evident, a great attention has been focused on some new natural sources of antimicrobial agents, including mushrooms. Medicinal mushroom species mostly belong to lignicolous (wood-decaying) fungi and represent particularly promising organisms with bioactive compounds acting as antimicrobials.

#### Objectives

In the present study, the genotoxic effect of 80% ethanol and 70% methanol, extracts of some lignicolous edible and medicinal fungal species: *Agrocybe aegerita*, *Meripilus giganteus*, *Coriolus versicolor*, *Daedaleopsis confragosa*, and *Stereum fuscum* were investigated. *Ganoderma applanatum* extract was prepared in ethanol after hydrodistillation procedure. They have been tested against bacteriophage DNA ( $\delta$  - Podoviridae and  $\sigma$ -1 - Siphoviridae), as well as against DNA extracted from *Escherichia coli* (ATCC 25922) and *Micrococcus flavus* ATCC 40240.

#### Methods

The extracted DNAs from viruses and bacteria were analyzed after treatment with fungal extracts (initial concentrations 200 mg/ml and 500 mg/ml) after 24 h in broth medium. Activity of the analyzed extracts on DNA fragmentation was visually determined on agarose gel after electrophoresis.

#### Conclusions

After 24h, extracts of *C. versicolor* and *G. applanatum* lead to the disintegration of *E. coli* DNA, while the extract of *M. giganteus* showed slight effect. All extracts showed disintegration effect against both phage DNAs, except extract of edible species *A. aegerita*. The other extracts give effect in concentration 1:100, except *S. fuscum* and *C. versicolor* against  $\sigma$  -1 phage DNA. The phage DNAs were not disintegrated with

any extract in concentration 1:10 000. The results showed that some higher concentrations of extracts possess genotoxic activity.

## FEMS-3003

### Fungal bacterial interactions

#### ANTIBACTERIAL AND GENOTOXIC ACTIVITY OF FOMES FOMENTARIUS (L.) FR. AND PLEUROTUS OSTREATUS (JACQ.) P. KUMM. FUNGAL EXTRACTS

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#### Background

Since fungi share common microorganisms such as *E. coli*, *S. aureus* and *P. aeruginosa* with animals and humans, it is believed that the humanity will meet more benefits from fungi and mushrooms, as antibiotics producers, than from plants. Many lignicolous species have been detected to act as antimicrobials until now, but activities are mostly influenced by fungal strain specificities and habitat characteristics, including geographic position.

#### Objectives

Since antibiotic mechanisms of fungi are still unclear, the aim of this investigation was to find connection between antibacterial and DNA genotoxic activity. Two kinds of extracts were prepared from fresh samples of autochthonous fungal species: *Fomes fomentarius* and *Pleurotus ostreatus* after simple extraction and after pouring with liquid nitrogen in order to disintegrate sporoderm.

#### Methods

Antibacterial testing was done with 70% MeOH extracts using macro and micro-dilution methods. Genotoxic effect against two bacterial strains *E. coli* and *M. luteus* was checked with 20%, 10% for *P. ostreatus* and 10%, 2.5% for *F. fomentarius* extract concentration. After 24h incubation, isolated DNA was applied on agarose gel electrophoresis.

#### Conclusions

The best antibacterial activity was obtained for *F. fomentarius* extracts mostly against Gram positive strains: *M. flavus*<sup>ATCC40240</sup>, *S. aureus*<sup>ATCC25923</sup>, *C. perfringens*<sup>ATCC19404</sup> and *B. subtilis*<sup>ATCC6633</sup> and gram negative *E. coli*<sup>ATCC25922</sup>. Significant increase in the size of bacterial cells was detected.

The MIC showed significant *E. coli* DNA fragmentation, indicating that this can be a possible mechanism of antibacterial activity, but also that these extracts can have a potential genotoxic activity which should be further examined.



**FEMS-1892**

## **Fungal bacterial interactions**

### **ENHANCED ACCESS TO ORGANIC NITROGEN AS A DIRECT BENEFIT IN THE INTERACTION OF THE FUNGUS MORCHELLA CRASSIPES WITH SOIL BACTERIA**

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#### **Background**

Fungi and bacteria have a long co-evolutionary history in terrestrial ecosystems. In soils, they are the basis for nutrient recycling. Therefore, not only do they co-exist, but they are also potential competitors for resources. In our laboratory, it was shown that the bacterium *Pseudomonas putida* benefits from interaction with fungi by using the so-called “Fungal Highways” to disperse in porous unsaturated soil-like media. Dispersal allows the bacteria to reach nutrient sources, as their mobility is limited in these conditions. However, the direct fitness benefit for the transporting fungi remains unknown. Until now, a long-term benefit was shown for the ectomycorrhizal and saprophytic fungus *Morchella crassipes*, which acted as a farmer of the dispersing bacteria and storing bacterial carbon into resting structures.

#### **Objectives**

We focus on the investigation of the benefit obtained by fungi during dispersal of bacteria.

#### **Methods**

Experiments were all undertaken on skimmed-milk agar medium (SMA; protein as N source) to see how extracellular proteolytic activity is used to access organic nitrogen. In addition, the fungal enzymes responsible were characterized according to their activity.

#### **Conclusions**

We have determined that fungal proteolysis can be enhanced by the presence of *P. putida* even when fungal cells are removed. The enhanced activity requires living bacterial cells, which is also observed for other bacteria dispersing in the fungal

mycelia. In conclusion, enhancement of proteolytic activity seems to be a direct fitness benefit for the interaction of *M. crassipes* with soil bacteria.



**FEMS-2451**

**Fungal bacterial interactions**

## **ENDOPHYTIC BACTERIA OF TOMATO: A SOURCE OF ANTIFUNGAL AND ANTIBACTERIAL COMPOUNDS**

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### **Background**

Many microorganisms are found inside various plant tissues. There, they not only compete with the plant immune system, but also with other endophytes and pathogenic microorganisms. We study endophytes of two underexplored above ground niches inside the tomato plant, the xylem and the leaf apoplast. Xylem vessels are the habitat of vascular wilt causing pathogens such as the bacterium *Clavibacter michiganensis* and the fungus *Verticillium dahliae*, whereas the leaf apoplast harbours the leaf mould *Cladosporium fulvum* and the late blight oomycete *Phytophthora infestans*.

### **Objectives**

Study the mechanism of *in planta* inter-microbe competition. Identify biocontrol species

### **Methods**

Endophytic bacteria were isolated from tomato xylem and leaf apoplastic space. Colonies were picked after incubation on TSA for 2-10 days at 25°C. Isolated strains were inoculated on a lawn of fungal, bacterial or oomycete plant pathogens and the release of antimicrobial compounds was scored. Garden cress was inoculated with selected strains. Seedling development was followed, and after 20 days endophytic bacteria were re-isolated from the stems.

### **Conclusions**

We isolated >120 tomato associated bacteria. More than half have antifungal, antibacterial and/or anti-oomycete properties during *in vitro* co-culture. >75% of species isolated from above ground parts of tomato were gram-positive. Garden cress was inoculated with strains with strong antifungal properties. Most strains promoted plant growth, and could be re-isolated from the stems, indicating a true endophytic lifestyle. Currently we are elucidating the antagonistic mechanisms and testing their potential for inhibiting pathogens *in planta*. In conclusion, the above

ground tomato endophytic bacteria are a promising source of antimicrobial compounds.

**FEMS-2937**

**Fungal bacterial interactions**

## **EFFECT OF BACTERIAL DISPERSAL ON THE BIOAVAILABILITY OF SEMIVOLATILE ORGANIC CONTAMINANTS**

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### **Background**

Contaminants bound to biogeochemical interfaces (BGI) are only hazardous if they become bioavailable. Therefore, the most effective remediation technique should lead to an optimal coverage of BGI with degrading microorganisms in order to prevent contaminant release to the environment. Simultaneously they should reduce the contaminant concentration at its source.

### **Objectives**

Here, we investigated the impact of bacterial dispersal on BGI on the outgassing of semivolatile phenanthrene (PHE). We tested the hypothesis whether the presence of previously described fungal transport networks ('fungal highways') of bacteria leads to: (i) bacterial distribution along the transport network, (ii) efficient bacterial distribution on the surface, and (iii) an increased biomass production fostering the degradation of PHE, that are released from the system.

### **Methods**

We designed a laboratory microcosm that mimicked a continuous PHE release to a model surface (agar) in the presence and absence of model dispersal networks (glass fibers) that facilitated the transport of the poorly motile PHE-degrading *Pseudomonas fluorescens* on agar surfaces.

### **Conclusions**

We observed that the presence of the dispersal network resulted in (i) an increased spatio-temporal spreading of bacteria, (ii) an increased bacterial coverage and growth on the agar surface, and (iii) a subsequent effective degradation of outgassing PHE and effective reduction of PHE contamination beyond the PHE hotspot. Our data suggest that fungal mycelia may promote the formation of an adapted microbial population that will degrade hazardous molecules that desorb from the contaminant source. Potentially, such an activity produce no emission of contaminants to the pore and groundwater, and hence, to higher organisms.

**FEMS-3088**

**Fungal bacterial interactions**

**STUDYING INITIAL FUSARIUM GRAMINEARUM-BIOCONTROL PSEUDOMONAS FLUORESCENS INTERACTIONS IN AN ESTABLISHING MICROTITER PLATE MICROCOSM SYSTEM**

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**Background**

The hyphosphere constitutes a microenvironment in which enzymes, metabolites and antibiotics secreted by the fungi are abundant which could attract bacteria.

**Objectives**

We here describe the first steps in establishing a microcosm system to study fungal-bacterial interaction under low-nutrient conditions emulating natural habitats like soil or the rhizosphere, then study fungal-bacterial interactions. *Fusarium graminearum* head blight in cereals was used as a model fungus, whereas *Pseudomonas fluorescens* strains P13, UTPF105, UTPF127, UTPF125 and B4 identified as biocontrol agents from Iranian soils were used as model bacteria.

**Methods**

For establishing this microcosm, 1000 spores of *F. graminearum* with 100 µl diluted medium was added to each microtiter well and incubated for 38 h at 20°C then the media was removed and inoculated with 100µl of 10<sup>4</sup>/ml bacterial suspension. After 24 h further incubation the microcosm system was stained by SYBR Green (SG) and Calcofluor white (CW) fluorescent stains. SG binds DNA and absorbs blue light and emits green light ( $\lambda = 520\text{nm}$ ). CW binds to chitin. Some photos of each CW (under UV light) and SG (under blue light) wells were taken. Both blue hyphae and green DNA signals were analyzed by ImageJ software. The experiment was arranged as randomized complete design with 4 replications. A Duncan test with a probability of 0.01 was used to show significant differences between treatments.

**Conclusions**

CW result showed that the biocontrol strains decreased fungal growth. In this reduction, UTPF125 was more successful. SG results showed that in a same hyphosphere, growth of different bacterial strains were significantly different.

**FEMS-2469**

**Fungal development**

## **A MECHANISM OF ALTERNATIVE SPLICING IN FILAMENTOUS FUNGI**

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### **Background**

Alternative intron splicing could lead to frame-shifted ORFs so that peptides with different terminal extensions can be produced. Alternative splicing of introns bordered by coding exons may yield the presence or absence of additional domains. We have found a *Penicillium chrysogenum* gene that yields two transcripts of different size.

### **Objectives**

An alternative intron gives rise to the two mature mRNAs using alternative, coupled lariat-branchpoint domain/acceptor sites. In *P. chrysogenum*, the short variant is 213 nt, the long variant is 891 nt long. Both transcripts yield a full-length protein. We found the presence of one canonical donor and one canonical lariat-branchpoint domain/acceptor couple within the short variant of the alternative intron, potentially defining an “internal” intron of 64 nt. We investigated whether this “internal” intron produces splicing intermediates.

### **Methods**

By sequencing cloned RT-PCR amplification products, we have elucidated the sequences of the mature mRNAs and that of splicing intermediates in *P. chrysogenum* and *Aspergillus nidulans*.

### **Conclusions**

We showed that contrary to “intron definition theory”, the “external” (5') donor was not used with the most 5' (“internal”) acceptor site. The “internal” (3') donor was not used with the most distal (most 3') acceptor site. It is the “internal” intron that is spliced alternatively using the 3' donor and either of the closely neighbouring lariat-domain/acceptor couples. The identity of the (more outward) acceptor used for the subsequent removal of the “external” intron and hence, the peptide ultimately produced, are thus primarily determined by the outcome of the first, alternative splicing reaction.



**FEMS-1430**

**Fungal development**

## **REGULATION OF THE CUTINOLITIC SYSTEM IN ASPERGILLUS NIDULANS**

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### **Background**

Cutinases play a role in plant pathogenesis as they degrade cutin, a structural lipid component of plant cuticle. They also act on short- and long-chain fatty acids, a feature that makes them suitable for biotechnology applications (Carvalho C, 1998). In *Aspergillus nidulans* genome there are four putative genes of cutinases (ancut1,2,3, and 4) and neither their role in cutin degradation or the expression conditions and their regulation are well understood, although it may be related to lipid metabolism (Hynes MJ, 2006).

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### **Objectives**

- Determine the expression levels of the cutinases encoded in the *A. nidulans* genome in response to different carbon sources that may act as repressors or inducers of the enzymes.
- Study the effect of two global TF (farA, farB) involved in lipid metabolism over the cutinase expression.
- Propose a regulation model of the cutinolytic system.

### **Methods**

*Aspergillus nidulans* was grown in minimal medium and different carbon sources were used. Mycelium was used for RNA's extraction with TRIzol. cDNA was synthesized using dT primers and Reverse Transcriptase. Finally the expression level of the genes was measured in Multiplex qRT-PCR assays and normalized with an endogenous gene. These expression levels were compared with the enzyme activity found in the supernatants in all the conditions studied.

## Conclusions

*ancut1*, *ancut2* and *farA* were upregulated with cutin and cutin monomers and showed CCR in presence of glucose. *ancut3* and *farB* were expressed constitutively and *ancut4* wasn't detected in any condition. Cutinase expression in *A. nidulans* resembles that found for *F. solani* (Li D, 1997).



**FEMS-1070**

**Fungal development**

**RNA-MEDIATED SILENCING OF A GENE ENCODING FOR A ZN(II)<sub>2</sub>CYS<sub>6</sub> PROTEIN CHANGES THE PHENOTYPE OF PENICILLIUM ROQUEFORTI**

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**Background**

The fungus *Penicillium roqueforti* has been poorly studied. Currently, we are studying a strain of this fungus that contains a dominant active Gai protein. This strain shows several phenotypic alterations compared with the wild-type strain. In a preliminary study, we observed a cDNA sequence differentially expressed in the wild-type strain. This sequence matched an unnamed ORF from *P. roqueforti* that encodes a putative Zn(II)<sub>2</sub>Cys<sub>6</sub> protein of unknown function. Proteins containing Zn(II)<sub>2</sub>Cys<sub>6</sub> domains are exclusively found in fungi, but few of them have been functionally characterized.

**Objectives**

To characterize a gene from the filamentous fungus *Penicillium roqueforti* that encodes a Zn(II)<sub>2</sub>Cys<sub>6</sub> protein, whose function to date remains unknown. We have named this gene *pcz1*.

**Methods**

*pcz1* was silenced using RNAi-silencing technology. Two transformants (named M9 and M11) showed dramatic reductions in the levels of *pcz1* transcript, confirming the successful knockdown of the gene. In these transformants, the apical extension rates, the conidiation, and the conidial germination were measured by standard methodologies.

**Conclusions**

The silencing of *pcz1* in the transformants altered the normal phenotype of *P. roqueforti*. **Specifically**, RNA-mediated silencing of *pcz1* resulted in the strong repression of conidiation, decreased apical growth, and the promotion of conidial germination in *P. roqueforti*, even in the absence of a carbon source. Our results suggest that *pcz1* may be involved in fungal development. Specifically, it may be a positive regulator of conidiation and apical growth, but it may be a repressor of conidial germination in *P. roqueforti*.

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**FEMS-0839**

**Fungal development**

**PROMOTER ANALYSIS OF AN ACIDIC LACCASE GENE EXPRESSED BY  
EXTERNAL PH IN AN INKY CAP, COPRINELLUS CONGREGATUS**

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**Background**

*Coprinellus congregatus*, generates mushrooms from dikaryotic mycelia, and this fungus produces several laccases: hyphal tip laccase and primordial laccase and they are not secreted outside of cells. When the dikaryon was grown in an acidic medium (pH 4.0-4.5), a new laccase was secreted into the culture supernatant. We have also cloned its genomic and cDNA genes, and also confirmed its expression only under acid condition by Northern blot analysis.

**Objectives**

There must be two responsive regions in the promoter region which are related to the signals that the mycelia is a dikaryon and is under acid stress respectively. In order to determine the responsive domain in the promoter, a recombinant construct having the acidic laccase gene promoter and green fluorescent protein (GFP) gene was generated.

**Methods**

The construct was introduced into *C. congregatus* monokaryones (a1 and a2), and the transformants were selected. Heterozygotic and homozygotic transformants were generated by mating proper partners. These transformants were transferred to the acid liquid media. Fungal cells were collected and they were used for the analysis of the promoter expression under confocal microscope and/or real time RT-PCR.

**Conclusions**

Homozygotic transformants showed more than 25 times higher expression than the negative control: this system helped the promoter domain analysis. Different lengths of acid laccase gene promoter-GFP will be constructed, and these will be introduced to the monokaryons. When the transformants carrying these various lengths of promoter

were analyzed at the acid culture conditions, the responsible doamins for dikaryon and acid stress will be determined.

**FEMS-3043**

**Fungal development**

## **TRANSCRIPTIONAL LANDSCAPE OF A MODEL MUSHROOM SCHIZOPHYLLUM COMMUNE**

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### **Background**

Transcription start site (TSS) sequencing gives a high resolution map of transcription initiation region for identifying promoters and fine-tuned transcriptional regulation mechanism. Although TSSs of various eukaryotic model organisms have been revealed, no species belonging to Basidiomycota has been known yet.

### **Objectives**

In order to understand transcriptional regulation in terms of promoters and transcriptional regulation on growth conditions, we report the first TSS analysis of Basidiomycota, the lignin-degrading fungus *Schizophyllum commune*

### **Methods**

Total 8.5 million RNA reads were generated by rapid amplification of cDNA ends (RACE) from two different growth conditions (cellulose or glucose as a sole carbon source) and mapped into the genome to allocate the transcription start sites.

### **Conclusions**

The positions of transcript head for the 4,959 genes were retrieved from both growth conditions. Comparing to the features of promoters found in eukaryotic model organisms, TSS distribution of *S. commune* were categorized into distinct groups such as single start sites ('sharp' promoter), multiple start sites ('broad' promoter) or mixed. To associate these features with DNA motifs in promoters, we examined the upstream 500 bp region of all genes. We found 16 motifs in the 'sharp' promoter upstream regions including typical TATA-box. The TSS analysis provides not only a basis for understanding of promoter structures but also a principle to build synthetic promoter construction for desired gene expression in *S. commune*.

**FEMS-1332**

**Fungal development**

### **THE PREVALENCE OF PNEUMOCYSTIS JIROVECII IS CHANGED?**

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#### **Background**

*Pneumocystis pneumonia* (PCP) that is caused by *Pneumocystis jirovecii* is prevalent in HIV-infected patients. Pneumocystis colonization of *Pneumocystis* is associated with airway inflammation and obstruction.

#### **Objectives**

The current study was conducted to identify the prevalence of *P. jirovecii* in suspected tuberculosis (TB) patients and four HIV positive patients in Ahvaz, Iran.

#### **Methods**

111 suspected TB positive patients and four HIV patients were investigated for *P. jirovecii* identification from Bronchoalveolar lavage specimens. The specimens were subjected for Grocott's methenamine silver (GMS) staining and then DNA extraction following Nested-PCR with specific primers. The PCR product then subjected for sequencing.

#### **Conclusions**

The findings demonstrated 50% of HIV positive patients were contaminated with *P. jirovecii* and 2 remaining patients showed negative results. In 111 suspected TB positive patients 25.2% were positive for TB, which 9 samples were positive for PCP by *P. jirovecii*. In negative TB patients, 19 positive *P. jirovecii* were identified. The analysis by Chi-square demonstrated that there was no significant difference between sex and presence of *P. jirovecii*. The current statistical analysis also presented no significant difference between TB and *P. jirovecii*. Finally, the GMS only detected *Pneumocystis jirovecii* in three patients. Our findings demonstrated high prevalence of *P. jirovecii* in Ahvaz which was more than the previous reports in Iran. Our investigation revealed that the *Pneumocystis jirovecii* is important in all respiratory tract infected patient not only in HIV or TB patients.

**FEMS-1044**

**Fungal development**

**AXL2, THE HOMOLOGUE OF THE YEAST BUD SITE SELECTION PROTEIN  
AXL2P AFFECTS ARTHROSPORE FORMATION IN ACREMONIUM  
CHRYSOGENUM**

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**Background**

*Acremonium chrysogenum* is the natural producer of the  $\beta$ -lactam antibiotic cephalosporin C. Due to its antibacterial activity against gram-positive and gram-negative bacteria, this  $\beta$ -lactam antibiotic is of great biotechnological and medical relevance. To optimize cephalosporin C production efficiently, continuous and directed improvement of industrial strains is required.

**Objectives**

A typical morphological feature of *A. chrysogenum* is the fragmentation of vegetative mycelium into arthrospores. These are uni- or binuclear cells, which develop during a prolonged cultivation under limited nutrient supply. Due to the known correlation of cephalosporin C production and arthrospore formation, we are interested in the identification of specific regulatory factors affecting both, cephalosporin C biosynthesis and morphological development. Here, we present a functional characterization of Axl2 in *A. chrysogenum*, a homologue of the yeast bud site selection protein Axl2p. In addition to that we are interested in the localization of Axl2 and other proteins of the bud site selection complex to verify their direct regulation of arthrospore formation.

**Methods**

Homologous recombination through split-marker approach; microscopic analysis

**Conclusions**

An *axl2* deletion strain of *A. chrysogenum* shows an accelerated arthrospore formation in comparison to its recipient strain. Therefore it might act as putative regulator of septation processes of fungal hyphae and consequently affect the arthrospore formation. To further investigate this developmental process we were able to establish the visualization of filamentous actin with the live cell marker Lifeact.



**FEMS-1621**

**Fungal development**

**STUDY OF COLONIZATION MECHANISMS OF CONSTRUCTION AND DECORATION PRODUCTS BY MICROMYCETES APPLIED TO THE DEVELOPMENT OF A PREVENTIVE TREATMENT**

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**Background**

In nature, saprotrophic fungi have an essential ecological role in decomposition of organic matter. In soils, they break down material like leaves, wood, dead bodies, etc. Fungal colonization in natural environments requires advanced functional relationships, including various antagonisms and synergisms, between the different components of the biotope. Colonization can be described, analyzed at different scales (macroscopic and molecular) and modeled. In indoor environment, fungi find niches favorable to their development, but are most often undesirable. Indeed, micromycetes induce pathologies to the occupants (allergies, poisoning and infections) and can also cause irreversible damages to the materials they infest. Colonization of different man-made substrates (construction and decoration products) by mold is insufficiently described to develop colonization models: Is artificial substrate colonization done serendipitously or is it determined like in natural environment by a defined succession of fungi driven by antagonisms and synergisms? A better knowledge of the dynamic of fungal colonization would be helpful to imagine preventive strategy for health and artifacts preservation.

**Objectives**

In this context, our research in microbial ecology aims to describe and understand the fungal colonization mechanisms in enclosed spaces.

**Methods**

First, a macroscopic characterization of the fungal succession on two artificial substrates has been made by culture and molecular methods. Secondly, Volatile Organic Compounds (VOC's) emitted by microfungi according to the growth substrates have been explored.

**Conclusions**

This new knowledge will offer alternatives to slow and limit fungal growth on building products to the use of traditional biocides.



**FEMS-1746**

**Fungal development**

## **IDENTIFICATION OF A SECOND LACTOSE PERMEASE GENE (LACPB) IN ASPERGILLUS NIDULANS**

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### **Background**

For most micro-organisms, lactose is a gratuitous carbon source that is slowly assimilated. In the filamentous fungus *Aspergillus nidulans*, lactose hydrolysis occurs intracellularly. Transport of lactose, which was previously shown to involve the lactose permease LacpA, is the limiting step in its catabolism in *A. nidulans*. Since *lacpA* deletion mutants are still able to grow on lactose, we assumed the existence of a second, physiologically relevant uptake system.

### **Objectives**

Identification and characterization of the second lactose permease gene (*lacpB*, locus AN2814) in *A. nidulans*, which encodes a structural paralog of LacpA.

### **Methods**

Single (*lacpB*) and double (*lacpA*, *lacpB*) lactose permease deletion mutants were created to test their phenotype (both growth and carbon source uptake) on solid and liquid media. Transcription profiles of *lacpB* and *lacpA* were also compared using Northern blot analyses.

### **Conclusions**

Growth of *lacpB*-single and *lacpA/lacpB* double deletants was strongly reduced on lactose on solid media, while in submerged cultures, the lactose phenotype was even more pronounced. Expression of the two *lacp* genes differed markedly. In addition to lactose, *lacpA* transcription also responded to D-galactose and L-arabinose, while *lacpB* was found induced by cellobiose, sophorose as well as lactose. Neither of the genes appeared to be expressed on D-glucose, D-xylose or D-mannose. We conclude that *lacpA* and *lacpB* are jointly responsible for wild-type lactose uptake by *A. nidulans*.

**FEMS-1690**

**Fungal development**

**CONIDIAL ANASTOMOSIS TUBES AND HETEROKARYOSIS IN THE ASEXUAL PLANT PATHOGENIC ASCOMYCETE VERTICILLIUM DAHLIAE**

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**Background**

*Verticillium dahliae* is a ubiquitous soilborne fungus that is known to reproduce strictly asexually. Therefore, different strains can only exchange and recombine genetic information through the parasexual cycle, starting with fusion (anastomosis) between hyphae of different genotypes to the formation of heterokaryons. Recently it was shown that parasexuality may alternatively begin in other fungi with fusion between conidial germlings via conidial anastomosis tubes (CATs).

**Objectives**

(i) To explore the possibility of self-/non-self fusion between *V. dahliae* germlings via CAT formation and study CATs' physiology, (ii) to study nuclear dynamics and heterokaryon formation through CATs, (iii) to identify signaling pathways that are required for CAT formation in *V. dahliae*.

**Methods**

(i) Live-cell imaging and analysis of fixed germling preparations with differential interference contrast (DIC) and fluorescence microscopy, (ii) tagging of fungal nuclei with fluorescent proteins for the study of nuclear migration, (iii) use of specific inhibitors of major signaling components and generation of corresponding gene deletion mutants via *Agrobacterium tumefaciens*-mediated transformation.

**Conclusions**

Typical CAT formation is frequent between *V. dahliae* germlings, although it is inhibited by the presence of nutrients and varies significantly between different strains. It is also dependent on pH, conidial density, and the history of the fungal cultures used. Cytoplasmic mixing and nuclear migration are permitted through CATs, allowing the formation of heterokaryons when conidia of different genotypes are combined. The formation of CATs in *V. dahliae* requires functional MAP kinase and ROS signaling components, similarly to normal development/morphogenesis and pathogenicity of the fungus.

**FEMS-1365**

**Fungal development**

**CO<sub>2</sub> REPRESSION OF FRUITING BODY FORMATION IN SCHIZOPHYLLUM COMMUNE IS MEDIATED VIA cAMP**

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**Background**

The most important environmental cues impacting fructification in *Schizophyllum commune* are light and CO<sub>2</sub>. Blue light is needed to initiate fructification, while high levels of CO<sub>2</sub> repress this process. The most ubiquitous mechanism of CO<sub>2</sub> sensing in prokaryotes and eukaryotes is based on cellular levels of cAMP. Regulation occurs through synthesis and degradation by adenylate cyclase (AC) and phosphodiesterase (PDE2), respectively. Most ACs localize to membranes and are activated by G proteins. However, there are also soluble ACs that are activated by bicarbonate or calcium ions. It has been proposed that fungal ACs are a hybrid between these two types and in most fungi there is only one gene for this protein.

**Objectives**

To study the mechanism for CO<sub>2</sub> sensing and its effect on fruiting body formation in *S. commune*.

**Methods**

cAMP was added to medium to assess its involvement in CO<sub>2</sub> sensing and fruiting in *S. commune*. Additional copies of *pde2* were introduced by transformation and verified by qPCR. IBMX was used to repress PDE activity.

**Conclusions**

Addition of cAMP to the medium inhibited fructification but did not impact growth. Inhibition of fruiting body formation could be counteracted by overexpressing *pde2*. The *pde2* overexpressor also fructified under repressing CO<sub>2</sub> levels where the wild type strain could not, reinforcing the existence of a link between CO<sub>2</sub> and cAMP levels. Addition of IBMX, an inhibitor of PDE2, to the medium abolished the effect of *pde2* overexpression. Taken together, these results show that CO<sub>2</sub> levels are sensed via cAMP.

**FEMS-0677**

**Fungal development**

**FUNCTIONS OF AUTOPHAGY-RELATED GENE SMATG12 IN FRUITING-BODY DEVELOPMENT OF SORDARIA MACROSPORA**

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**Background**

In eukaryotes, autophagy is a highly conserved degradation process by which cytoplasm including organelles is non-selectively engulfed by double-membrane vesicles called autophagosomes. After fusion of the outer membrane with the vacuole/lysosome a vesicle surrounded by the inner membrane, the autophagic body, is degraded by hydrolytic enzymes and building blocks are recycled into the cytoplasm

**Objectives**

In filamentous fungi, autophagy functions as a catabolic mechanism to overcome starvation conditions and to control diverse developmental processes under normal nutritional conditions. We recently showed that in the homothallic ascomycete *Sordaria macrospora*, autophagy related genes encoding components of the conjugation systems are required for fruiting body development or are essential for viability. Here, we cloned and characterized the *S. macrospora* (*Sm*)*atg12* gene, encoding a ubiquitin-fold protein.

**Methods**

To examine the role of *Smatg12* in detail, we replaced it with a hygromycin resistance cassette and generated a homokaryotic  $\Delta$ *Smatg12* deletion strain. GFP-labeled SmATG12 was used to localize the protein *in vivo*.

**Conclusions**

The  $\Delta$ *Smatg12* mutant displayed a decreased vegetative growth rate and was unable to form fruiting bodies. GFP-labeled SmATG12 was detected as punctured structures and co-localized with DsRED-SmATG8 at some places in the cytoplasm. We could show that lipidation of SmATG8 and proper autophagosome formation depends on SmATG12.

**FEMS-1345**

**Fungal development**

**ENZYME ACTIVITY AND MALDI TOF/TOF MS ANALYSIS OF RESPONSES TO STRESS INDUCED BY ARSENIC AND ANTIMONY IN ACIDOMYCES ACIDOPHILUS FROM TIN-MINING SOIL**

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**Background**

An *Acidomyces acidophilus* strain has been isolated from a tin mining soil in Cornwall, England that contained approximately 18970 mg kg<sup>-1</sup> arsenic (As) and 228 mg kg<sup>-1</sup> antimony (Sb) with the soil pH around 1. Culture experiments showed that the strain could tolerate up to 22500 mg kg<sup>-1</sup> of As(V) and 100 mg kg<sup>-1</sup> of Sb(V).

**Objectives**

This study examines the protein responses of *A. acidophilus* when exposed to As/Sb using MALDI TOF/TOF.

**Methods**

The strain was exposed for 24 h to 100 mg/L of sodium arsenate (Na<sub>2</sub>HAsO<sub>4</sub>) and potassium antimonate [KSb(OH)<sub>6</sub>]. MALDI TOF/TOF MS analysis was carried out and tentatively identified that the following proteins were up-regulated in response to Na<sub>2</sub>HAsO<sub>4</sub> and KSb(OH)<sub>6</sub>: malate dehydrogenase, phospholipase B, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase and 3-isopropylmalate dehydrogenase. In addition, the activities of enzymes glutathione reductase, glutathione transferase, superoxide dismutase and catalase in the presence of As and/or Sb are still being investigated. Also, the isolated *A. acidophilus* and three *A. acidophilus* reference type strains (CBS 335.97, CCF4251 and CCF3679) were characterised by MALDI TOF/TOF and all four strains showed very distinctive spectra that distinguish them readily. It has also been found that *A. acidophilus* grown in different media exhibited different mass spectra, thus affecting the identification of this strain.

**Conclusions**

This provides further and in-depth opportunity to understand the extreme resistance to heavy metals by *A. acidophilus* and also identify the enzymes that are involved in the resistance mechanisms and thus contain biotechnological potential and future scientific uses such as bioremediation and clinical studies.

**FEMS-1583**

**Fungal development**

**THE 7-TRANSMEMBRANE RECEPTOR GPR1 GOVERNS GROWTH, DEVELOPMENT AND MYCOPARASITISM-RELATED FUNCTIONS IN THE FUNGUS TRICHODERMA ATROVIRIDE AND INTERACTS WITH A SUR7 FAMILY MEMBRANE PROTEIN**

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**Background**

Species of the fungal genus *Trichoderma* are among the most successful biofungicides in today's agriculture although our understanding of the exact molecular mechanisms of their activity still is fragmentary. The biological control of plant diseases by *Trichoderma* includes direct antagonism of phytopathogenic fungi by parasitism. This mycoparasitic attack comprises sensing of the prey and chemotropic growth towards it followed by activation of "molecular weapons" such as cell wall-lytic enzymes, secondary metabolites, and infection structures. Consequently the receptors and signaling pathways involved in sensing and responding to the prey fungus are of special interest.

**Objectives**

The *Trichoderma atroviride* Gpr1 7-transmembrane receptor has been shown to be essential for regulating mycoparasitism-relevant processes and it is implicated in the recognition of prey-derived signals. For further characterization of Gpr1, respective target genes being regulated by the Gpr1 receptor upon prey recognition have been identified by comparative genome-wide microarray-based transcriptomics of *T. atroviride* wild-type as well as *gpr1* mutants.

**Methods**

Furthermore, direct interactors of Gpr1 were identified by the split-ubiquitin yeast-based two-hybrid system.

**Conclusions**

Functional characterization of one of the identified interactors, a plasma membrane protein belonging to the fungal-specific Sur7 family, by gene deletion revealed a role in the regulation of conidiation, polar growth, and the mycoparasitic attack of the prey fungus.



**FEMS-3118**

**Fungal pathogenicity in humans**

## **RAPID DETECTION OF CANDIDA SPECIES, CAUSING OCULAR INFECTION IN PAKISTANI POPULATION**

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### **Background**

The present study was conducted on fungal pathogen (*Candida*) causing ocular infections. The basis for effective treatment is rapid diagnosis of the disease and identification of its causative agent. Due to the limitations of classical methods for the detection of systemic fungal infections, it has become essential to develop a quick, sensitive and specific detection assay.

### **Objectives**

A polymerase chain reaction (PCR) method was to be optimized that would be capable of detecting a wide range of medically important fungi from clinical specimens.

### **Methods**

Eight *Candida species* sequences were downloaded from GenBank or EMBL databases. A set of unique primers were designed based on the conserved region in the given fungal species. To verify the specificity of the designed primers, the BLAST program at the NCBI website was employed to search the primers in short, near exact sequences. These primers were then analyzed by the AmplifX tool.

### **Conclusions**

The results indicated that this study gives the strategy for the rapid detection of *Candida* species on the basis of genotype. A reproducible method for genotypic identification of *Candida species* will be very helpful for the doctors/practitioners to detect the specific species. In current medical facilities in Pakistan, the prediction of *Candida sp.* involved in ocular infection will be a valuable addition of information in the field of medicine. Pharmacists can make different antibiotics against different



diseases related to fungus *Candida* and can easily find out the specific cause of the related disease.

**FEMS-0926**

**Fungal pathogenicity in humans**

**TRANSCRIPTIONAL PROFILE OF FUSARIUM OXYSPORUM AND FUSARIUM SOLANI CHALLENGED WITH ANTIFUNGAL COMPOUNDS IN VITRO**

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**Background**

*Fusarium* is a genus of filamentous fungi widely distributed and of high importance because several species of the genus are pathogens of plants and humans. In humans *Fusarium* is associated with superficial mycoses as onychomycosis and fungal keratitis in immunocompetent patients. However in immunocompromised patients, the infection can be disseminated and can cause the death of the patient due to the high resistance to antifungal treatments

**Objectives**

This project aimed to analyze the gene expression profile of both *Fusarium oxysporum* and *Fusarium solani* after the exposure to Posaconazole and Amphotericin B

**Methods**

The expression profile was performed by RNA-Seq of *Fusarium* spp and the genes with a significant expression were validated by qRT-PCR. We found an average of 20-30 of differentially expressed genes of the exposed strains relative to the controls

**Conclusions**

Some genes were associated with oxidative stress, ergosterol synthesis and cell wall structure, among others. The role of these genes in resistance against antifungal compounds is discussed. This study contributes to the general knowledge and understanding of the resistance mechanisms of *Fusarium* spp, which could improve the implementation of treatment strategies.

**FEMS-0784**

**Fungal pathogenicity in humans**

**ANALYSIS OF GENE EXPRESSION PROFILES IN *M. FURFUR* USING NEXT GENERATION**

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**Background**

The *Malassezia* genus is characterized by its lipophilic or lipid dependent species. These traits have been related with virulence, but until now the relation has not been clearly elucidated. *M. furfur* is a species presenting a high level of phenotypic variation. We previously described an atypical isolate of this species, which only assimilates tween 80. This finding suggested host adaptations mechanisms.

**Objectives**

We used microarray analysis to characterize the genetic expression that could give more clues about this metabolic advantage.

**Methods**

We analyzed gene expression differences between *M. furfur* CBS 1878 and the atypical *M. furfur* strain. In those experiments we used Dixon modified agar with and without tween 80. cDNA microarrays were designed and fabricated by Roche Nimblegen (NimbleGen Systems Inc., Madison, WI), using the *Malassezia globosa* genome. Normalization of the intensity values was performed using the VSN method. Differential expression analysis of the profiles was performed using pairwise comparisons and two different methods SAM and Ebayes algorithms. The enriched genes were analyzed by Gene Ontology (GO) terms.

**Conclusions**

The genes over expressed were associated with cyclophilins and thioredoxin, proteins involved in important cellular processes. We validated these results by qPCR. This analysis suggested that these candidate genes could be related with virulence.

**FEMS-1913**

**Fungal pathogenicity in humans**

**ANTIFUNGAL SUSCEPTIBILITY TESTING OF YEASTS ISOLATED FROM THE PATIENTS WITH NOSOCOMIAL INFECTION IN SLOVAKIA.**

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**Background**

Yeasts are common fungal opportunistic pathogens in humans playing a significant role in the morbidity and mortality of immunocompromised patients.

**Objectives**

The aim of the study was antifungal susceptibility testing of yeasts isolates to fluconazole, voriconazole, caspofungin, and anidulafungin.

**Methods**

From January 2013 through October 2014, 3200 isolates were obtained from hospitalized patients with nosocomial yeast infection. The distribution of the species obtained was *Candida albicans* (75.4%), *C. glabrata* (8.2%), *C. tropicalis* (4.8%), *C. kefyr* (3.4%), *C. parapsilosis* (2.5%), *C. lusitanae* (1.9%), *C. famata* (1.4%), *C. krusei* (1.4%), *C. guilliermondii* (1%), *C. sake* (1%), *T. asahii* (1%) and *T. mucoides* (1%). MICs of antifungal agents were determined by Etest method.

**Conclusions**

All the isolates of *C. albicans* were susceptible to the fluconazole. Higher voriconazole MICs were observed in isolates exhibiting higher fluconazole MICs, frequently in *C. krusei* and *C. glabrata*. Isolates with echinocandins MICs suggesting reduced susceptibility were only sporadic cases.

**FEMS-0526**

**Fungal pathogenicity in humans**

**VICISSITUDES IN ADHESION TO DENTURE ACRYLIC SURFACES OF ORAL CANDIDA DUBLINIENSIS ISOLATES FOLLOWING BRIEF EXPOSURE TO SUB-CIDAL CONCENTRATIONS OF POLYENES, ECHINOCANDINS, AZOLES AND CHLORHEXIDINE GLUCONATE**

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**Background**

Adherence of *Candida* to denture acrylic surfaces is implicated as the initial process in the pathogenesis of *Candida*-associated denture stomatitis. This condition can be treated with antimycotic agents belonging to polyene, echinocandin and azole groups of antimycotics. In addition chlorhexidine is also used in its management. In the niches of the mouth the intraoral concentration of these drugs fluctuates considerably due to the dynamics of the oral environment and *Candida* may undergo only to a brief exposure to therapeutic agents. It has been suggested that *Candida dubliniensis* may play an important role in the establishment and persistence of *C. dubliniensis* induced-denture stomatitis.

**Objectives**

Hence, the effect of brief exposure to sub-lethal concentrations of nystatin, amphotericin B, caspofungin, ketoconazole, fluconazole and chlorhexidine gluconate on adhesion of these isolates to denture acrylic surfaces was investigated.

**Methods**

After determining the minimum inhibitory concentration of the drugs, twenty oral isolates of *C. dubliniensis* were exposed to sub-lethal concentrations of these six drugs for one hour. Subsequently, the drugs were removed by dilution and the adhesion of these isolates to denture acrylic strips was assessed by an *in vitro* adhesion assay.

**Conclusions**

Compared to controls, exposure to nystatin, amphotericin B, caspofungin, ketoconazole, fluconazole and chlorhexidine gluconate suppressed the ability of *C. dubliniensis* isolates to adhere to denture acrylic surfaces by 74.68%, 74.27%, 73.33%, 57.31%, 44.57% and 56.53% ( $P < 0.001$  for all drugs), respectively. Hence, brief exposure to sub-lethal concentrations of antimycotics seems to exert an antifungal effect by suppressing the adhesion to denture acrylic surfaces of *C. dubliniensis* oral isolates.

**FEMS-1439**

**Fungal pathogenicity in humans**

**IDENTIFICATION OF EXTRACELLULAR ASPERGILLUS SPP COMPONENTS THAT INTERACT WITH IMMUNE RECEPTORS.**

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**Background**

*Aspergillus* species are worldwide distributed and ubiquitous in the environment, among this genus relevant industrial and medical relevant species are present. For instance, *A. niger* is used in industrial production systems and has also been reported to cause infections. Extracellular compounds from *Aspergillus* spp can play various roles during human infections, for example by modulation of immune system response.

**Objectives**

Identify novel extracellular fungal components that interact with immune receptors.

**Methods**

Culture supernatants from various *A. niger* strains (WT and mutants strains) were screened via a *Tricolor Assay* enabling identification of components that interact with cellular immune receptors. Briefly, the culture supernatant was first co-incubated with a human immune cell mix (neutrophils, monocytes and lymphocytes). Subsequently, fluorescently labeled immune receptor antibodies were incubated with the pre-incubated supernatant-cells mix in a 96-well plate. Subsequently, the plate was centrifuged, washed and cells were fixed. Finally, antibody-antigen interactions were measured using FACS analysis

**Conclusions**

We did not detect extracellular components in supernatants of *A. niger* wt (N402) that interact with immune receptor. However, a derivative of this *A. niger* wt strain, the mutant D15#26, did produce such components and which appear to be small since a 3 kDa filtrated supernatant was still active. This *A. niger* mutant strain lacks extracellular proteases due to a PrtT mutation. However, it also contains additional undefined mutations due to UV mutagenesis and which for example prevents acidification of the medium. We are characterizing this mutant to identify the components and genes that are involved.

## **FEMS-2638**

### **Fungal pathogenicity in humans**

#### **B39 - INVESTIGATION OF DERMATOLOGICAL SPECIMENS FOR SUPERFICIAL MYCOSES**

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#### **Background**

The term ‘superficial mycoses’ refers to fungal infections usually confined to the outer layers of the skin, hair and nails. Superficial fungal infections are chronic and recurring conditions and are caused by dermatophytes as well as non-dermatophytes.

Infection by dermatophytes is cutaneous and generally restricted to the non-living cornified layers in patients who are immunocompetent. They are generally unable to penetrate tissues which are not fully keratinised. Reactions to infections can range from mild to severe depending upon the host’s immune response, the virulence of the infecting species, the site of infection and environmental factors. The dermatophytes are classified in three genera: *Epidermophyton*, *Microsporum* and *Trichophyton* species.

There are non-dermatophytes that can infect healthy skin, nails damaged by physical trauma, or pre-existing infection with a dermatophyte.

#### **Objectives**

Aimed at practising professionals in the field of microbiology, B39 is a diagnostic tool which describes and recommends the procedures that can be used to visualise and isolate dermatophytes, non-dermatophytes and other fungi from skin, nail and hair specimens.

B39 is developed by professionals in the field with specialist input and is intended as a general resource for laboratory staff, clinicians and healthcare commissioners. This document focuses on how detection and accurate identification can aid the management and control of superficial mycoses.

#### **Methods**

-

#### **Conclusions**



B39 provides guidance on the best minimum practice when investigating infection caused by dermatophytes, non-dermatophytes and other fungi. It provides information on rapid molecular methods and culture testing for these fungi and aims to help drive pathology modernisation by recommending new technologies.

**FEMS-1479**

**Fungal pathogenicity in humans**

**ANTIFUNGAL ACTIVITY AND REDUCTION OF ERGOSTEROL CONTENT OF TRICHOPHYTON RUBRUM CAUSED BY GLYCOALKALOID ALFA-SOLANINE**

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**Background**

The dermatophyte *Trichophyton rubrum* is the most common causative agent of dermatophytosis in the world. Due the prominence of dermatophytosis and its socio-economic consequences, there is a need to develop of new antifungal agents. Most of the currently available drugs presents side effects and are ineffective against resistant strains of fungi. The plants are a significant source of biologically active natural products and many chemical structures are models for the synthesis of new drugs. The  $\alpha$ -solanine is a glycoalkaloid extracted from potato sprouts (Solanaceae) and has several biological activities, including antimicrobial activity.

**Objectives**

The aim of this study was to determine the minimum inhibitory concentration (MIC) of  $\alpha$  solanine and to quantify the ergosterol content of *T.rubrum* exposed to natural compound  $\alpha$ -solanine.

**Methods**

The susceptibility assay was performed using the microdilution test of  $\alpha$ -solanine and control fluconazole in the range of to 1000-1.9 $\mu$ g/mL toward the strain ATCC MYA-3108 of *T. rubrum*. Ergosterol content was extracted from mycelia exposed for 48 hours to MIC concentration of antifungals and was quantified by spectrophotometry.

**Conclusions**

The  $\alpha$ -solanine compound showed a MIC of 3.12  $\mu$ g /mL and fluconazole showed a MIC of 63  $\mu$ g/mL toward the strain ATCC MYA-3108. The  $\alpha$ - Solanine and fluconazole reduced 35% and 40.95% of ergosterol content, respectively. The  $\alpha$ -solanine showed significant activity toward *T.rubrum* and reduced the level of ergosterol of cell membrane, which is a specific target of the fungal cell.

**FEMS-2340**

**Fungal pathogenicity in humans**

**MOLECULAR IDENTIFICATION OF CANDIDA SPECIES ISOLATED FROM PATIENTS WITH VULVOVAGINAL CANDIDIASIS IN IRAN**

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**Background**

Candidal vulvovaginitis is an infection of the vagina's mucous membranes by *Candida* species. Up to 75% of women will have this infection at some point in their lives, and approximately 5% will have recurring episodes. It is the second most common cause of vaginal inflammation after bacterial vaginosis and usually caused by *C. albicans* but other species, including *C. glabrata*, *C. tropicalis*, *C. krusei* and *C. africana*.

**Objectives**

The aim of the present study was to molecular identification of *Candida* species isolated from patients with vulvovaginal candidiasis.

**Methods**

A total of 150 patients refer to Mazandaran University of Medical Sciences from 2013-2014. The vaginal swab specimens were cultured on Sabouraud Dextrose Agar (SDA) and identified based on conventional methods (Germ Tube Test, Chlamydospore production) and subsequently confirmed by PCR-HWP1 (produces 3 different DNA fragments: approximately 780 bp for *C. africana*, 941 bp for *C. albicans* and 250 bp for *C. glabrata*).

**Conclusions**

The current study shows that *C. albicans* as predominant species isolated from vulvovaginitis. It can be concluded that molecular diagnostic methods are reliable and would be useful for identification of medically important *Candida* species in clinical samples. Therefore considerable attention has been paid to prevention and treatment of microbial growth, which has resulted in improvement of patient management. Although recently molecular approaches have been developed for the identification and discrimination of *Candida* species, novel techniques for the diagnosis based on species level have been established in routine laboratories.

**FEMS-1278**

**Fungal pathogenicity in humans**

## **BURDEN OF CANDIDA NON ALBICANS ISOLATES WITH A HIGH ANTIBIOTIC RESISTANCE PATTERN CAUSED OROPHARYNGEAL CANDIDIASIS AMONG CANCER PATIENTS**

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### **Background**

*Candida albicans* is a frequent component of oral ecology found in up to 75% of humans; particularly among immunocompromised patients. *Candida* species can cause a multitude of disease ranging from mild oral disease to disseminated candidiasis.

### **Objectives**

In this study, an attempt has been made to determine susceptibility pattern of four antifungal agents against the *Candida* species isolated from cancer patients with oropharyngeal candidiasis.

### **Methods**

Isolation and identification of *Candida* spp was done based on standard procedures. Antifungal resistance pattern was carried out according to CLSI guideline. Identification of *Candida* species were confirm using the 18s Ribosomal RNA.

### **Conclusions**

Leukemia and lymphoma were the most frequent cancer in the studied group, accounting for 17 (34%) and 12 (24%) respectively. A total of 29 *Candida* spp were isolated from 29 of cancer patients; of which 17 were *C. albicans* and 12 were *C. non – albicans*. All the *Candida* spp were confirmed using the 18s Ribosomal RNA. Among all the *Candida* spp, *Candida non – albicans* showed a high resistance pattern to amphotericin B (MIC 07 µg / ml) and ketokenazole (MIC=05 µg / ml). In conclusion, oropharyngeal Candidiasis is a serious infection among cancer patients. The isolated *candida* spp were resistant to common antifungal agents which may leads to longer hospital stay, more expensive/ toxic drugs and higher mortality. Therefore, interval surveillance is necessary in developing institutional guidelines.



**FEMS-3073**

**Fungal pathogenicity in humans**

**XANTHOMEGNIN DETECTION DOES NOT DISCRIMINATE BETWEEN TRICHOPHYTON RUBRUM AND T. MENTAGROPHYTES COMPLEXES**

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**Background**

High-performance liquid chromatography (HPLC) is a reliable and robust methodology in medical diagnostics, including the detection of hemoglobin variants, vitamin D levels, and *Trichophyton rubrum* in skin and nail materials. Previous information reported that the latter was reliably identified using xanthomegnin activity via HPLC.

**Objectives**

In the present study, we aimed to discriminate between the two most common human pathogenic dermatophytic fungi, *T. rubrum* and *T. mentagrophytes* complexes, via the detection of xanthomegnin using HPLC methodology.

**Methods**

Hence, we investigated 32 reference dermatophyte strains: *Arthroderma* spp. ( $n=7$ ), *T. rubrum* ( $n=18$ ), and *T. mentagrophytes* complex ( $n=7$ ) strains. In addition, we analyzed both clinical samples and strains from 13 samples identified as *T. rubrum* ( $n=9$ ) and *T. interdigitale* ( $n=4$ ) using ITS-sequencing. Twelve of 18 reference *T. rubrum* complex strains produced detectable amounts of xanthomegnin. Moreover, and more importantly, 2 *A. vanbreuseghemii* and 1 *T. interdigitale* reference strains also demonstrated xanthomegnin activity. Furthermore, from a total of 13 clinical samples, 11 clinical samples and 11 clinical strains were positive for xanthomegnin, including *T. interdigitale* strains.

**Conclusions**

Although xanthomegnin detection is a rapid and inexpensive method, we concluded that it is not a reliable method for discriminating between *T. rubrum* and *T. mentagrophytes* complexes given that only two-thirds of the reference *T. rubrum* complex strains and 3 strains from the *T. mentagrophytes* complex produced xanthomegnin activity. In addition, we believe that the results of the present study should be of interest to scientists and clinicians specifically in the field of medical mycology.

**FEMS-2573**

**Fungal pathogenicity in humans**

**INTERACTIONS BETWEEN HUMAN EXTRACELLULAR MATRIX PROTEINS AND CANDIDA GLABRATA CELL WALL, STUDIED BY CHEMICAL CROSSLINKING AND MASS SPECTROMETRY**

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**Background**

*Candida glabrata* causes almost 20% of all fungal infections in humans. This yeast is distantly related to other medically important *Candida* species (such as the most widespread fungal pathogen *C. albicans*) and significantly differs from those species in the pathogenicity profile. One of essential steps in the microbial colonisation of human organism is the adherence of pathogen to host cells and the supporting extracellular matrix (ECM).

**Objectives**

*C. glabrata* cell surface is involved in initiating the contact between fungus and host during infection. The current study aims at identifying the major cell wall components that interact with human ECM proteins.

**Methods**

*C. glabrata* cell wall-associated proteins were isolated with  $\beta$ -1,3- or  $\beta$ -1,6-glucanases and allowed to interact with biotin-labelled fibronectin, vitronectin and laminin. The formed protein-protein complexes were fixed by chemical crosslinking with a heterobifunctional, photoactivatable reagent, sulfosuccinimidyl 2-[(4,4'-azipentanamido)ethyl]-1,3'-dithiopropionate. The stably coupled proteins were adsorbed on streptavidin-conjugated beads and identified by tandem mass spectrometry.

**Conclusions**

Binding of fibronectin, vitronectin and laminin to *C. glabrata* cell surface was confirmed and several fungal proteins involved in these interactions were identified. Some of them are common binders for all three investigated ECM proteins and predominantly belong to a group of loosely bound with the cell surface, cytoplasm-derived proteins. Examples of these ECM-binding proteins include enolase, alcohol dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and malate synthase.

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**FEMS-2785**

**Fungal pathogenicity in humans**

**USE OF PCR-RFLP AND PCR-HWP1 FOR IDENTIFICATION OF CANDIDA SPECIES ISOLATED FROM CYSTIC FIBROSIS PATIENTS.**

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**Background**

Due to the predisposing conditions in patients with cystic fibrosis (CF) caused by defective mucociliary clearance facilitates of colonization and invasion with *Candida* species has dramatically increased. Molecular techniques utilizing amplification of target DNA provide quick and precise methods for diagnosis and identification of *Candida* species.

**Objectives**

The aim of current study was identification of the most medically common isolated *Candida* species from the air way of CF patients by PCR-RFLP and amplification of HWP1 gene.

**Methods**

A total of 42 CF patients presenting symptoms referred to pediatric respiratory diseases research center were screened for the presence of *Candida* spp. The isolates initially were phenotypically identified and confirmed by molecular approaches based on restriction fragment length polymorphism (PCR-RFLP) for discrimination of *C. albicans* of non *albicans* and amplification of HWP1 gene for discrimination of *C. albicans* from *C. dubliniensis* and *C. africana* was conducted.

**Conclusions**

Results show that *C. albicans* was the most frequently isolated species (83.8%) followed by non-*albicans* included *C. parapsilosis* (7.1%), *C. glabrata* (3.2%), and *C. tropicalis* (3.2%). The restriction patterns of each *Candida* species were perfectly specific. Since *MspI* is not able to discriminate between three morphological similar species, *C. albicans*, *C. dubliniensis* and *C. africana*, we used PCR amplification of *hwp1* gene, which (7.1%) species from *C. albicans* identified as *C. dubliniensis*, however *C. africana* strains were not found. It can be concluded that molecular diagnostic methods are reliable and would be useful for identification of medically important *Candida* species in clinical samples.

## FEMS-1317

### Fungal pathogenicity in humans

#### NEWLY IDENTIFIED CAUSAL AGENTS OF FUNGAL KERATITIS

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#### Background

Fungal keratitis is a serious suppurative, usually ulcerative corneal infection which may result in blindness or reduced vision.

#### Objectives

Our aim was to study the spectrum of fungi causing keratitis in South India based on the data collected at the Aravind Eye Hospital and Postgraduate Institute of Ophthalmology, Coimbatore, Tamilnadu between 2005 and 2014.

#### Methods

Fungal strains were isolated from corneal scrapings of keratitis patients. Morphological examination of the isolates and nucleotide sequence-based identification were performed.

#### Conclusions

The most frequently occurring genus was *Fusarium*. Representatives of five *Fusarium* species complexes were detected. Members of *Fusarium solani* species complex (FSSC) were the most frequent, followed by the species complexes *Fusarium dimerum* (FDSC), *Fusarium fujikuroi* (FFSC), *Fusarium oxysporum* (FOSC) and *Fusarium incarnatum-equiseti* (FIESC). *Fusarium napiforme* from FFSC and the sexual fungus *Neocosmospora vasinfecta* from FOSC were firstly detected from keratitis. *Aspergillus* proved to be the second most frequent genus with *Aspergillus*

*flavus* as the predominant species, followed by *Aspergillus fumigatus* and *Aspergillus terreus*. *Aspergillus tamarai*, *Aspergillus nomius* and *Aspergillus pseudotamarai* from section *Flavi*, *Aspergillus tubingensis*, *Aspergillus brasiliensis*, *Aspergillus neoniger* and *Aspergillus welwitschiae* from section *Nigri*, *Aspergillus sydowii* and *Aspergillus variegator* from section *Nidulantes*, *Aspergillus amstelodami* from section *Aspergillus*, *Aspergillus melleus* from section *Circumdati* and *Aspergillus lentulus* from section *Fumigati* of the genus were firstly recognized as potential causal agents of fungal keratitis.

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**FEMS-2470**

**Fungal pathogenicity in humans**

**FUGEMIA IN HOSPITALIZED PATIENTS WITH PROLONGED FEVER WITH BACTEC METHOD**

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**Background**

fungemia is a very important infection in terms of incidence and mortality. It can be caused by several species of the genus *Candida* and should be diagnosed and treated quickly

**Objectives**

This study was performed to assess prevalence of fungemia in patients with prolonged fever using two blood culture method

**Methods**

This descriptive study was carried on 330 blood samples obtained from patients with prolonged fever despite widespread antibiotic in Beheshti hospital of Kashan using both BHI and BACTEC blood culture within 2010-2013

**Conclusions**

Among 330 patients with prolonged fever, 16 cases of fungemia were detected which all of them were positive in BACTEC method and 12 were positive in BHI method. All of the isolated fungus were *Candida* and the most frequent agent of candidemia was *Candida Albicans*. The predisposing underlying factors were malignancy, diabetes, using of widespread antibiotics and corticosteroid, trauma and surgery. The rate of fungemia was 4.8% (16/330). We found *Candida Albicans* as the most common causative agent of candidemia in our samples.

**FEMS-2630**

**Fungal pathogenicity in humans**

### **CASE SERIES OF RHINOCEREBRAL MUCORMYCOSIS**

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#### **Background**

Rhinocerebral mucormycosis (RCM) is a rare, fulminating opportunistic fungal infection caused by a fungus of order Mucorales

#### **Objectives**

The purpose of this paper is to draw attention to the clinical presentation of RCM and to emphasize need for high index of suspicion in diagnosis and treatment

#### **Methods**

This was a case series study conducted in Beheshti Hospital of Kashan, from 2001 to 2013. The charts of identified patients were reviewed for the following information: demographics, predisposing conditions, anatomical location of infection, treatment, length of hospital stay

#### **Conclusions**

Cases were between 21 to 88 year old. The most patient were female (sex ratio: 2). The clinical sign and symptoms of patients were hematemesis, epistaxis, melena, headache, pain, swelling and tenderness on the face and paranasal sinuses, malaise and weakness, fever, blurred vision, periorbital edema, black secretion and necrosis of nasal septum or hard palate, cranial nerve palsy. All the patients had history of diabetes. CT scan of PNS revealed consolidation, increased thickness, necrosis and disruption of mucosal wall of sinuses. Amphotericin B was prescribed for all the patients. The length of hospital stay was more than 20 days. Blindness occurred in one patient. 3 patients died and the rest recovered

The diagnosis of rhinocerebral mucormycosis should be considered in the clinical setting of necrotic sinusitis in diabetic patients. Early diagnosis and treatment are crucial factors leading to a good outcome.

**FEMS-2275**

**Fungal pathogenicity in humans**

**BGL2P FROM SACCHAROMYCES CEREVISIAE CELL WALL – THE HOMOLOG OF CANDIDIASIS BIOMARKER FROM CANDIDA ALBICANS IS ABLE TO FORM FIBRILS**

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**Background**

The most common fungal infections in humans are candidiasis caused by *Candida* yeast. A major, highly conserved yeast cell wall (CW) protein Bgl2p, which can also be secreted into the growth medium, plays a significant role in candidiasis. It was shown that the injected into a mouse's bloodstream *C.albicans* with *BGL2* deletion possessed decreased infectivity compared to the one without *BGL2* deletion (Sarthý et al., 1997). On the other hand, high level of antibodies against Bgl2p is prognostic factor, which defines the risk of mortality (Pitarch et al., 2006). Authors proposed to use Bgl2p from *C.albicans* for vaccine development. Earlier we demonstrated that Bgl2p from *S.cerevisiae* CW (Bgl2p homolog from *C.albicans*) could reveal amyloid properties (Kalebina et al., 2008). At the beginning of our work the process of Bgl2p amyloid formation remained unclear.

**Objectives**

It was suggested that Bgl2p as well as many other proteins of microorganisms appeared as a virulence factor due to its amyloid forming ability. In order to verify it we needed to study whether Bgl2p was able to form fibrils under conditions similar to those of a bloodstream. Primarily we were interested in investigating the influence of two factors on Bgl2p fibrillation: the pH value within a neutral range and the presence of regulatory molecules such as polyphosphates.

**Methods**

We investigated Bgl2p from non-pathogenic *S.cerevisiae* as a model using fluorescence confocal and electron microscopy, fluorescence spectroscopy, bioinformatics analysis.

**Conclusions**

Bgl2p fibril formation ability was strongly regulated by pH value (Bezsonov et al., 2013), polyphosphates, divalent cations and buffer components. The results obtained allowed us to conclude that Bgl2p might exist in fibrillary form under bloodstream-like conditions. The described Bgl2p amyloid formation ability is important for further fighting the candidiasis including vaccine development, using Bgl2p from non-pathogenic *S.cerevisiae*.

FEMS-2122

## Fungal pathogenicity in humans

### ANALYSIS OF THE PROFILE OF PARACOCCIDIODES SPP. IN RESPONSE TO THE DRUG BY TRANSCRIPTIONAL/PROTEOMIC APPROACHES AND IDENTIFICATION OF COMPOUNDS WITH ANTIFUNGAL POTENTIAL.

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### Background

*Paracoccidioides* spp, a complex of several phylogenetic species, is the agent of paracoccidioidomycosis (PCM). PCM is endemic in Latin America, with 80% of cases reported in Brazil, where it is the eighth-leading cause of mortality among infectious and parasitic diseases, establishing it as a serious public health problem. The use of small molecules of natural origin as medicines or as prototypes of drugs is a well established theme in the drugs development.

### Objectives

Argentilactone, bioactive metabolite isolated from *Hyptis ovalifolia* and inhibited the isocitrate lyase from *Paracoccidioides*. Thiosemicarbazide is a small molecule terpene camphene derivative that inhibits the growth of *Trichophyton mentagrophytes*. Indole alkaloids due their already described antifungal activity, we have included several carboline alkaloids isolated from *Rubiaceae* species in our screening antifungal program aiming to discover compounds with potential to inhibit malate synthase from *Paracoccidioides*.

### Methods

In this study we performed the transcriptome, proteome analysis and virtual screening of *Paracoccidioides* spp. yeast cells growth in the presence of compounds and/or itraconazole and amphotericin B.

### Conclusions

A total of 1.058 genes were identified, of which 208 were up-regulated and 850 down-regulated in argentilactone response. Four indol alkaloids showed ability to reduce the *PbMLSr* activity and inhibit the adhesion of the fungus and *PbMLS* to extracellular matrix components. In our proteomic analyzes various classes of proteases were suppressed in the presence of thiosemicarbazide, were identified and sequenced 11

differentially expressed proteins in the condition with itraconazole, and 29 for the condition treated with amphotericin B.



**FEMS-0585**

**Fungal pathogenicity in humans**

## **ORAL MICROFLORA AND THEIR RELATION TO RISK FACTORS IN HIV+ PATIENTS WITH OROPHARYNGEAL CANDIDIASIS**

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### **Background**

Oropharyngeal candidiasis (OPC) is considered an opportunistic infection caused by a ubiquitous fungal organism that is routinely seen as normal oral flora.

### **Objectives**

The purpose of this study was to determine the prevalence of oral microflora and association of oral candidiasis and multiple risk factors in HIV<sup>+</sup> patients.

### **Methods**

The present study included 100 HIV-infected patients participated in Imam Khomeini Hospital, Tehran, Iran for OPC and HIV. Samples were obtained from the oral cavity and direct microscopic examination, gram staining and culture on standard microbiological media were performed in all patients. CD<sub>4</sub><sup>+</sup> cell count/CD<sub>4</sub><sup>+</sup> percentage were also calculated.

### **Conclusions**

A total of 460 bacterial colonies were obtained and *Streptococcus mutans* (15.4%) was the most frequently isolated species in the HIV<sup>+</sup> patients. In addition, 254 yeasts (from 4 different genera) were isolated from the patient under study. *Candida* species (94.4%) were the most frequently obtained genera, followed by *Saccharomyces* (2.4%), *Kluyveromyces* and *Cryptococcus* (1.6% for both) species. *Candida albicans* (37.2%) was the most common species isolated from HIV<sup>+</sup> patients with OPC. The mean CD<sub>4</sub><sup>+</sup> cell counts were 154.5 cells/μL, with a range of 8 to 611 cells/μL. Thirty percent (30%) patients had a CD<sub>4</sub><sup>+</sup> cell count between 101 and 200 cells/μL (28.7% of total yeasts isolated). Our results showed that yeasts of the genus *Candida* were isolated at a comparable rate from the oral cavity of HIV<sup>+</sup> patients and there was no significant difference of the variables CD<sub>4</sub><sup>+</sup> cell count and yeast counts.

**FEMS-0586**

**Fungal pathogenicity in humans**

**DIFFERENTIAL EXPRESSION OF THE VACUOLAR ASPARTIC PROTEINASE (APR1) GENE IN CANDIDA ALBICANS STRAINS ISOLATED FROM MALAYSIAN AND IRANIAN HUMAN AND MICE BLOOD**

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**Background**

Intracellular proteinase A is an aspartic enzyme in *Candida albicans* that is expressed by *APR1* gene.

**Objectives**

The aims of this study were to evaluate and to compare *APR1* gene expression in *C. albicans* strains isolated from Malaysian and Iranian patients and mice infected by *C. albicans* obtained from Malaysian and Iranian patients.

**Methods**

The evaluation of *APR1* gene expression was performed using reverse transcriptase-polymerase chain reaction (RT-PCR) with two-pair primers and 18S rRNA as housekeeping gene. The expression of *APR1* gene and 18S rRNA was determined on agarose gel electrophoresis.

**Conclusions**

The results did not show any significant difference in *APR1* gene expression between *C. albicans* isolated from mice blood infected by *C. albicans* isolated from Malaysian and Iranian patients ( $p>0.05$ ). The expression of *APR1* gene in *C. albicans* strains isolated from Malaysian patients was more than Iranian patients ( $p<0.05$ ). In addition, the gene expression in *C. albicans* strains obtained from mice blood was significantly higher than *C. albicans* strains isolated from both Malaysian and Iranian patients ( $p<0.05$ ). Considering to the important role of intracellular proteinase A in *C. albicans*, *APR1* gene as producer of this enzyme can act as an important gene in virulence of *C. albicans* in different environmental situations.

**FEMS-0587**

**Fungal pathogenicity in humans**

**ANTIFUNGAL ACTIVITY OF ZATARIA MULTIFLORA, PLARGONIUM GRAVEOLENS AND CUMINUM CYMINUM ESSENTIAL OILS TOWARDS THREE SPECIES OF MALASSEZIA ISOLATED FROM PATIENTS WITH PITYRIASIS VERSICOLOR**

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**Background**

*Malassezia* species have been recognized as members of the microbiological flora of human skin that cause skin diseases such as pityriasis versicolor (PV).

**Objectives**

To investigate the anti-*Malassezia* activities of *Zataria multiflora*, *Plargonium graveolens* and *Cuminum cyminum* essential oils (EOs) against different pathogenic *Malassezia* species isolated from patients with pityriasis versicolor (PV).

**Methods**

The EOs were obtained by water-distillation using a Clevenger-type system. Anti-*Malassezia* activity against *Malassezia* species was carried out using disk diffusion method in Dixon agar.

**Conclusions**

The main oil components were carvacrol (61.3%) and thymol (25.2%) for *Z. multiflora*,  $\alpha$ -pinene (30%) and limonene (21%) for *C. cyminum* and citronelol (28.2%) and geraniol (22.1%) for *P. graveolens*. The three *Malassezia* species showed a similar susceptibility to the three plants tested, *C. cyminum* (mean value: 48.3 mm) being the most active, followed by *Z. multiflora* (mean value: 28.1 mm) and *P. graveolens* (mean value: 26.1 mm). This study indicated that *Z. multiflora*, *P. graveolens* and *C. cyminum* EOs have considerable anti-*Malassezia* activities, deserving further investigation for clinical applications for the treatment of PV.

**FEMS-1192**

**Fungal pathogenicity in humans**

**DELETION LIBRARY REVEALS POTENTIAL GENES INVOLVED IN THE VIRULENCE OF CANDIDA PARAPSILOSIS**

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**Background**

Since the 1980s, fungi have emerged as causes of human disease. Some fungal species are opportunistic pathogens, that are only able to cause diseases in individuals with damaged physical barriers or/and altered immune status. The incidence of *C. parapsilosis* caused candidemia has dramatically increased over the past decade, therefore, the understanding of the pathomechanism of this species becomes urgent.

**Objectives**

Despite the growth of genome sequence information, a large number of fungal genes remain uncharacterized. In our previous work several fungal transcriptional factors have been identified using RNA-Seq data that were over expressed during host-pathogen interactions. Based on these data we aimed to generate a knock out library to be able to analyze these genes individually.

**Methods**

Fusion PCR was applied to generate gene specific deletion constructions to disrupt genes from the genome of a *C. parapsilosis* double auxotrophic strain. The growth abilities of the null mutant strains were tested in different conditions such as different medias and temperatures along with survival in the presence of various stressors.

**Conclusions**

We have found null mutants that show differences in appearance such as increased pseudohyphae formation, regressed growth on different temperatures and alkali-phobic phenotype. Comparison of the virulence of these deletion strains using infectious models is now in progress. In the future with the use of this method we are able to identify key regulatory factors that may play a role in the virulence of *C. parapsilosis* during host-pathogen interactions.



## **FEMS-1720**

### **Fungal pathogenicity in humans**

#### **THE CFEM HEME-IRON ACQUISITION NETWORK IN PATHOGENIC FUNGI**

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#### **Background**

Iron acquisition represents a challenge for all organisms due to the low solubility of iron under oxidizing conditions. This challenge is particularly acute for pathogenic microorganisms, due to the iron withholding mechanisms deployed by the host. Many microbial pathogens have therefore evolved mechanisms to extract iron from hemoglobin, the largest iron store in the host, by removing the heme cofactor from the globins and transferring it to the microbe's cytoplasm. The fungal pathogen *Candida albicans* uses a family of conserved extracellular proteins for that purpose, the CFEM proteins <sup>1</sup>.

#### **Objectives**

. The cell wall-anchored CFEM proteins Rbt5 and Pga7 are both able to extract heme from hemoglobin <sup>2</sup>. The heme can then subsequently be transferred from one CFEM protein to the next <sup>2</sup> until it reaches the plasma membrane, whereupon it is endocytosed by a mechanism involving the ESCRT pathway <sup>3</sup>. A third, secreted CFEM protein, Csa2, is shown here to also participate in the heme-iron acquisition relay

#### **Methods**

. Biochemical and structural analysis

#### **Conclusions**

Biochemical and structural analysis of Csa2 reveals that CFEM proteins are unrelated, at either the sequence or structure level, to the known bacterial heme-scavenging proteins, suggesting that microbes have evolved several different independent solutions to the heme-iron acquisition problem.

**FEMS-0710**

**Fungal pathogenicity in plants**

**REGULATION OF BOTRYTIS CINEREA VIRULENCE GENES IN INTERACTION WITH TRICHODERMA ARUNDINACEUM IS MEDIATED BY THE SESQUITERPENE HARZIANUM A**

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**Background**

*Trichoderma* includes a great diversity of species, some of them with the ability to control the growth of fungal phytopathogens. Many of these strains produce secondary metabolites that are able to inhibit the growth of their fungal preys. However, pathogens can also produce metabolites that in some cases belong to the same family of those produced by *Trichoderma*.

**Objectives**

In the present work we study the interaction between Harzianum A (HA) produced by *T. arundinaceum* (Ta37) and botrydial (BOT) produced by *Botrytis cinerea*. HA exhibits antifungal activity, and BOT is a strong virulence factor in plant disease.

**Methods**

A procedure of confrontation between these two strains was implemented, followed by the analysis of gene expression by real-time PCR using mycelia isolated from the confrontation areas. To better characterize the regulatory responses produced by HA, the TaDTri5 mutant, isolated by disruption of *tri5* gene and blocked in the synthesis of HA, was also used.

**Conclusions**

It was observed that HA induced expression of genes involved in BOT biosynthesis when HA was added to *B. cinerea* growing alone. However, in confrontation experiments against TaDTri5 the expression of these genes was higher than in confrontation with Ta37, indicating that the mutant must produce some metabolite/s, responsible for such induction. A different metabolite production pattern was described for Ta37 and TaDTri5<sup>(a)</sup>, which is in agreement with the proteomic characterization of these two strains, suggesting that genetic disruption of *tri5* had a systemic effect of the fungal physiology not only restricted to the terpene pathway.

(a) Doi: 10.1111/1462-2920.12514.



**FEMS-1820**

**Fungal pathogenicity in plants**

## **CHANGES OF THE ALTERNATIVE OXIDASE ACTIVITY OF BOTRYTIS CINEREA ISOLATES UNDER VARIOUS CONDITIONS**

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### **Background**

*Botrytis cinerea* (teleomorph *Botryotinia fuckeliana* [de Bary] Whetzel) is the causal agent of grey mould on a wide range of crops. Some of the pesticides used for chemical control inhibit the respiration electron transport chain. Resistance develops rapidly due to the high genetic variability of *B. cinerea*. In addition, a functional alternative oxidase (AOX) may also play a role in the survival of the fungus. Expression of AOX is often increased under biotic and abiotic stress, providing the organism adaptation capacity to different environmental conditions. It may also provide the respiratory electron transport chain an extra capacity when the cytochrome pathway is inhibited or saturated.

*B. cinerea* includes two cryptic species: group I (*B. pseudocinerea*) and group II (*B. cinerea sensu stricto*), which could differ in their pathogenicity, fungicide resistance, host variability and genetic diversity.

### **Objectives**

In this study the differences between the alternative respiratory activity of *B. cinerea* belonging to group I and II were investigated. We also studied the effects of osmotic stress and the temperatures on the respiratory system.

### **Methods**

Measurements were carried out and analyzed with polarographic oxygen electrode system. The cytochrome pathway was inhibited with KCN while SHAM was used for blocking the alternative respiration.

### **Conclusions**

More intense alternative respiratory activity was observed in group II isolates under optimal growth conditions. The alternative pathway was more profoundly induced by osmotic and temperature stress compared to the cytochrome pathway, and this

correlated well with increased transcript formation of the *aox1* gene encoding the AOX enzyme.

**FEMS-1858**

**Fungal pathogenicity in plants**

**CHARACTERIZATION OF COMPONENTS PRODUCED BY AUREOBASIDIUM PULLULANS, ANTAGONISTIC AGAINST MANGO ANTHRACNOSE DERIVED BY COLLETOTRICHUM GLEOSPORIOIDES**

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**Background**

Mango anthracnose derived from *Colletotrichum gleosporioides* reveal severe symptoms in terms of surface pitting, skin browning in the surface of mango fruits.

**Objectives**

In this study, We explore to screen the antagonistic yeasts against mango anthracnose and study the antagonistic mechanisms and characteristics of the components produced by the antagonistic yeasts.

**Methods**

Seven strains of *C. gleosporioides* were isolated from 8 infected mangoes. On the pathogenic test, strain WU2L01-1 could infect mangoes and reveal pitting and skin browning as that of anthracnose, indicating the strain is a phytopathogen to mango.

**Conclusions**

Twelve of 578 yeasts exhibited antagonistic activity against *C. gleosporioides*. These strains were classified into the member of the genus *Aureobasidium*, *Cryptococcus* and *Jaminalia* species, based on the sequences of the LSU D1/D domain of rRNA gene. The antagonistic components were characterized by analyzing the activity of degradative enzyme, siderophore and glycolipid. All the experiments demonstrated that these strains cannot produce high activity of siderophore and degradative enzymes,  $\beta$ -1.3 -glucanase, chitinase, amylase, and ?. While, the extracts from culture broth derived from different yeast strains by methanol, ethyl acetate or dichloromethane were confirmed to have the antagonistic activity against *C. gleosporioides*. The optimum medium was tested and obtained for producing antagonistic components. Till now, strains NN5L07, NN5L08, NN9L05,

GY10L05 and HM4L10 and could produce higher productivity of antagonistic components based on these results.

**FEMS-1825**

**Fungal pathogenicity in plants**

## **AGROTRANSFORMATION SYSTEM APPLIED IN COLLETOTRICHUM CITRUS PATHOGEN**

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### **Background**

Brazil is responsible for a third of world production of orange, as well as for 80% of international orange juice market. The European Union is the main market in this Brazilian agribusiness. But, the citrus crop is affected by a range of pathogens that decrease the production and the business viability. One of these diseases is Postbloom Fruit Drop (PFD) caused by one fungal species from the *Colletotrichum acutatum* species complex, which causes serious losses in citrus fruit production. Many works are being developed to study and control this phytopathogenic fungus.

### **Objectives**

An alternative method to investigate the fungus infection process is the use of fluorescence protein.

### **Methods**

For that, we applied the agrotransformation system to transform a *Colletotrichum* isolate from citrus symptomatic blossom, proven as pathogenic.

### **Conclusions**

The methodology became efficient to insert genes in the fungus. The transformers have the ability to express the red fluorescent protein due the insertion of DsRed gene into the fungal genome. They were evaluated in fluorescence microscopy and is possible to identify spores, apressoria and fungus hyphae. Therewith, we efficiently applied the transformation methodology by *Agrobacterium tumefaciens* in the *Colletotrichum* citrus pathogen and these transformants can be used to infection process studies and evaluated for eventual pathogenic alterations as result of insertion mutagenesis.

**FEMS-0744**

**Fungal pathogenicity in plants**

## **ESTABLISHMENT OF GENETIC MANIPULATION SYSTEM FOR USTILAGO ESCULENTA**

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**Background:** *Ustilago esculenta* is a fungal endophyte that triggers stem swelling of *Zizania latifolia* resulting in an edible aquatic vegetable. To date, characterization of the interaction between *U. esculenta* and *Z. latifolia* remains descriptive due to a lack of genetic manipulation and re-infection strategies.

**Objective:** In this study, a transformation system for *U. esculenta* was developed based on methods developed for *U. maydis* and evaluated.

**Methods:** To improve protoplast yield, regeneration rate and transformation efficiency, we systematically adapted several factors, including enzyme digesting conditions, osmotic pressure stabilizers, vectors and antibiotics. The adapted transformation protocol was confirmed by knocking out the *UeICL* (coding isocitrate lyase) gene and over-expressing the *EGFP* gene. Correct transformants were detected by PCR and fluorescence microscopy analysis.

**Conclusions:** The protocol was optimized that 100 mg fresh fungus yielded the maximum number of protoplasts after digested for 3 hours at 30°C in 2 ml SCS solution containing 15 mg/ml Lywallzyme with the osmotic pressure stabilizer of 0.4 M sucrose. The protoplasts obtained highest regeneration rate when growing on Reg-light-Agar medium containing 0.8 M sucrose. *Bsa*I-mediated Golden Gate Reactions and counter-selection with hygromycin of 50 µg/ml or carboxin of 5 µg/ml were successfully used in constructing plasmids and identifying mutants. Similar to most filamentous fungi, flank length around 1kb with homologous ends was efficient for *U. esculenta*. Some sequences of *U. maydis*, like P<sub>otef</sub> and NLS (nuclear localization signal), worked well in *U. esculenta*. We successfully established a genetic manipulation protocol for *U. esculenta* through protoplast transformation.

**FEMS-2920**

**Fungal pathogenicity in plants**

**FIRST REPORT OF ANTHRACNOSE ON CASSAVA CAUSED BY *C. GLOEOSPORIOIDES* F. SP. *AESCHYNOMENE* IN THAILAND**

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**Background**

Anthrachnose cassava incited by *Colletotrichum* is a prevalent disease in cassava producing regions.

**Objectives**

The aim of this study was to identify the *Colletotrichum* associated with cassava in Thailand.

**Methods**

Cassava anthracnose symptoms (circular, sunken lesions with spore masses produced in black acervuli) were observed on cassava leaves in Nakhon Sawan, Thailand. Four single-spore isolates were derived from fungal cultures isolated from 4 different infected cassava samples.

**Conclusions**

Colonies grown on HPDA changed from white to grey with an average colony diameter of 8.45x8.60 cm after 7 days. Their conidia were cylindrical and 4.75x 14.00 µm in size. To test the four isolates for pathogenicity, after 3 days, typical anthracnose symptoms had developed on the inoculated cassava leaves but not on that of the control treatment. The pathogen was re-isolated from the inoculated cassava but not from the control cassava. Based upon these morphological and cultural characteristics, the 4 isolates were initially identified as *C. gloeosporioides* f. sp. *aeschynomene*. To confirm identity of the four isolates, their internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA), were amplified with ITS3 and ITS4 universal primers. The amplified DNA sequences of these isolates shared 99% homology with the ITS sequences of *C. gloeosporioides* f. sp. *aeschynomene* in the GenBank (JX131331, KC816043, JX139578 and JX258732). To our knowledge, this is the first report of *C. gloeosporioides* f. sp. *aeschynomene* causing anthracnose on cassava.

**FEMS-1381**

## **Fungal pathogenicity in plants**

### **PENJA PEPPER UNDER THREAT IN CAMEROON**

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## **Background**

“Penja Pepper” (*Piper nigrum* L.) is cultivated in the Penja region of Cameroon. It is famous for its exceptional aroma and taste. However, in Cameroon pepper is attacked by various diseases. Moreover, the support tree used for growing pepper vines in Cameroon, *Spondias mombin*, is also subject to disease attacks.

### **Objectives**

In order to maintain the viability of Penja Pepper cultivation, these diseases must be identified and controlled. The aim of this study was to identify the principal pathogens of black pepper vines and its support tree.

### **Methods**

Samples from pepper vines, *S. mombin* and the soil were collected from various fields in the Penja region. Using a combination of baiting and selective agar medium techniques several potential causal agents were isolated. The isolated microorganisms were identified based on morphology as well as ITS sequencing.

### **Conclusions**

Eight oomycete isolates were obtained from pepper and 18 basidiomycete isolates from either pepper or *S. mombin*. The eight oomycete isolates of Pepper were tentatively identified as *Phytophthora vexans* and/or *P. cucurbitacearum*. The morphological identification of the basidiomycete isolates show that it is an *Armillaria* sp. Pathogenicity tests were carried out with 180 days old pepper plants. The plants were inoculated with discs of mycelium introduced in the soil and on the collar. This is the first time that *Armillaria* is found to be pathogenic to pepper as well as its support



tree *Spondias mombin*. The importance of these finding for controlling these diseases to safeguard the sustainable production of the Penja Pepper of Cameroon is discussed

**FEMS-2403**

**Fungal pathogenicity in plants**

**CATALASE FROM BACILLUS SP. M10 AND ITS POTENTIAL BIOCONTROL ROLE IN THE PROPHYLACTIC AND SYMPTOMATIC TREATMENT OF THE TOMATO ANTHRACNOSE FUNGUS, COLLETOTRICHUM CAPSICI**

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**Background**

Anthrachnose which is caused by *Colletotrichum* spp. is the major disease of tomato crops around the world. Antifungal substances have been reported as biocontrol agents against several phytopathogenic fungi. Previously, *Bacillus* sp. M10 was found to inhibit the growth of several fungal pathogens of tomato including *Colletotrichum capsici*.

**Objectives**

To purify and identify the antifungal protein from *Bacillus* sp. M10, and subsequently determine the effects of the antifungal protein to the fungus and its ability to prevent and/or protect tomato fruits from the post-harvest anthracnose disease caused by *C. capsici*.

**Methods**

Ammonium sulfate precipitation and DEAE anion exchange chromatography were performed to purify the antifungal protein. Excised band from SDS-PAGE was analyzed by MALDI-TOF MS. Light and scanning electron microscopy were used to observe the effects of the protein to the fungus. Finally, prevention and protection of postharvest anthracnose disease using the antifungal protein were evaluated.

**Conclusions**

After 40–60% ammonium sulfate precipitation and DEAE anion exchange chromatography, the antifungal protein was enriched 11-fold and was found to retain a specific activity of 189.57 AU/mg. SDS-PAGE revealed that the purified protein has a molecular weight of ~55.4 kDa. MALDI-TOF MS of the excised, enriched protein band revealed the closest similarity to the vegetative catalase (KatA) from *Bacillus amyloquefaciens*. Microscopic observation showed that the protein induced abnormal hyphal elongation and conidial swelling and rupture. The protein caused a marked inhibition of anthracnose development on tomato fruits. Thus, this putative catalase may potentially be useful as a biocontrol agent against anthracnose disease.

**FEMS-2415**

**Fungal pathogenicity in plants**

### **AUXIN ROLE AND PRODUCTION IN CLAVICEPS PURPUREA**

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### **Background**

Biotrophic fungus *Claviceps purpurea* is known as a parasite of monocots, invading mainly rye florets (*Secale cereale*). The late infection is characteristic by the formation of dark purple sclerotia, which are the source of ergot alkaloids, substances used in pharmaceutical industry. During the infection, host plant does not show any symptoms of immune response and fungal hyphae grow unrecognized through the plant tissue. Plant defence responses are driven by phytohormone crosstalk and the fungus might utilize its own phytohormone biosynthesis for the host manipulation. Preliminary results showed that mycelium of *Claviceps purpurea* contains high amount of major auxin – indole-3-acetic acid (IAA).

### **Objectives**

The aim of this work is to characterize an auxin biosynthesis in *Claviceps purpurea* and to determine an auxin role in *Claviceps* infection strategy

### **Methods**

IAA concentration and its precursors and conjugates was analysed by TLC and HPLC coupled with the MS detection. Putative genes participating on different auxin biosynthesis pathways and translocation mechanism were deleted by the homologous recombination and transformants have been characterized upon the infection and in axenic cultures.

### **Conclusions**

Genome data mining and measurements of auxin biosynthesis intermediates identified two possible pathways of IAA biosynthesis in *Claviceps purpurea* based on plant and bacterial models. Detailed characteristic of several deletion mutants and WT showed interested features indicating that auxin is secreted from fungal tissue upon the infection and actively translocated in fungi itself.



**FEMS-2536**  
**Mycobacteria**

## **ROLE OF TETRAZOLIUM BROMIDE REDUCTION ASSAY IN DIAGNOSIS OF SUSPECTED CASES OF MULTIDRUG RESISTANT(MDR)PULMONARY TUBERCULOSIS**

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### **Background**

Early detection of multidrug resistant tuberculosis (MDR-TB) is of primary importance for both management & infection control. It is complicating TB control efforts in several low- & middle-income countries. Colorimetric method is one of the useful techniques for early detection of MDR-TB.

### **Objectives**

To evaluate drug susceptibility testing by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay and proportion method to diagnose suspected cases of MDR-TB.

### **Methods**

Sputum from 37 previously treated smear positive patients with pulmonary tuberculosis having clinical suspicion of MDR-TB were included in the study. Culture from Lowenstein-Jensen(LJ) media was inoculated into 7H9 broth and after 7days the inoculum was tested for isoniazid(INH) & rifampicin(RIF) susceptibility testing by MTT assay. The formation of formazan product after addition of MTT (after 7days) was taken as positive for *Mycobacterium tuberculosis*.

### **Conclusions**

In this study 18 cases were sensitive and 15 cases resistant to isoniazid by MTT assay. Twenty one cases were sensitive and 12 cases resistant by drug susceptibility testing of rifampicin by MTT assay. The sensitivity & specificity of MTT assay for INH was 90% & 100% and for RIF 90.90% & 90.90% respectively. The MTT assay is a simple, low cost susceptibility test that can be completed in approximately 7 days. Due to its high levels of agreement with proportion method, the MTT method has the potential to provide rapid detection of INH & RIF-resistant *M. tuberculosis* in clinical use.



**FEMS-1763**  
**Mycobacteria**

**ANTITUBERCULOSIS DRUG RESISTANCE PATTERNS OF MYCOBACTERIUM TUBERCULOSIS COMPLEX STRAINS ISOLATED WITHIN A SIX YEARS PERIOD IN A TERTIARY REFERRAL HOSPITAL IN ISTANBUL**

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**Background**

Drug resistance in tuberculosis (TB) is a growing global problem.

**Objectives**

The aim of this study was to determine the resistance rates of *Mycobacterium tuberculosis* complex (MTC) isolates to major antituberculosis drugs (ATBDs) (isoniazid, rifampicin, ethambutol, streptomycin) within six years period in a tertiary referral hospital.

	2009	2010	2011	2012	2013	2014
<b>Clinical Sample Counts</b>	1101	1243	1617	2025	1789	2346
<b>Isolate Count (%)</b>	82 (7,5)	50 (4)	25 (1,6)	33 (1,6)	56 (3,1)	34 (1,5)
<b>Resistant Isolates (%)</b>	26 (31,7)	22 (44)	5 (20)	11 (33,3)	13 (23,2)	6 (17,6)
<b>Single INH Resistance (%)</b>	11 (13,4)	7 (14)	1 (4)	5 (15,2)	4 (7,1)	1 (2,9)
<b>Single RIF Resistance (%)</b>	1 (1,2)	1 (2)	0 (0)	0 (0)	0 (0)	2 (5,9)
<b>Single EMB Resistance (%)</b>	3 (3,7)	7 (14)	0 (0)	0 (0)	0 (1,8)	0 (0)
<b>Single SM Resistance (%)</b>	0 (0)	1 (2)	1 (4)	0 (0)	2 (3,6)	2 (5,9)
<b>INH+RIF</b>	2 (2,4)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
<b>INH+RIF+SM</b>	0 (0)	1 (2)	1 (4)	3 (9,1)	5 (8,9)	0 (0)
<b>INH+RIF+EMB</b>	5 (6,1)	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
<b>INH+RIF+SM+EMB</b>	3 (3,7)	0 (0)	2 (8)	2 (6,1)	0 (0)	0 (0)
<b>Total MDR (%)</b>	<b>10 (12,2)</b>	<b>4 (8)</b>	<b>3 (12)</b>	<b>5 (15,2)</b>	<b>5 (8,9)</b>	<b>0 (0)</b>
<b>Total PDR (%)</b>	<b>1 (1,2)</b>	<b>2 (4)</b>	<b>0 (0)</b>	<b>1 (3)</b>	<b>1 (2)</b>	<b>1 (2,9)</b>

\* SM: Streptomycin, INH: Isoniazid, RIF: Rifampicin, EMB: Ethambutol, MDR: Multi-drug resistance, PDR: Poly-drug resistance

**Methods**

Clinical specimens were cultured and the susceptibilities of isolates to the major ATBDs were tested using the BACTEC MGIT 960 system. In a period of six years, 280 MTC strains are isolated from 10121 clinical samples. A decreasing trend was found on the MTC isolation rates from clinical samples with years (7.5% in 2009 to 1.5% in 2014). Of the 197 (70.4%) isolates were found sensitive to all four tested ATBDs and 83 (29.6%) of the isolates were found resistant to at least one ATBD.

Rate of single drug resistance for isoniazid was 10.4% (n=29), for ethambutol 3.9% (n=11), for streptomycin 2.1% (n=6) and for rifampicin 1.4% (n=4). Resistance rates of isoniazid, which was found as the most resistant drug among others, were decreasing (13.4% to 2.9%) while only rifampicin resistance was increasing (1.2% to 5.9%) by years. Rate of at least isoniazid and rifampicin resistant -multidrug resistant (MDR)- isolates was 9,6% (n=27). Number of MDR isolates was decreasing by years.

### **Conclusions**

Even though TB rates are decreasing by years, persistence of high isoniazid resistance rates and increasing resistance of rifampicin requires more strict applications of precautions and limitation of rifampicin use both for treatment and prophylactic purposes in other diseases than TB.



**FEMS-1800**  
**Mycobacteria**

**EXPLORING THE EVOLUTIONARY SUCCESS OF MYCOBACTERIUM  
TUBERCULOSIS BY INVESTIGATION OF RARE CLINICAL ISOLATES WITH  
SMOOTH COLONY MORPHOLOGY**

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**Background**

*Mycobacterium tuberculosis*, one of the most successful human pathogens, and other members of the *M. tuberculosis* complex (MTBC) show a mainly clonal population structure with 99.9% of genetic similarity. In contrast to these strains with predominantly rough colony morphology, rare clinical isolates with smooth morphology (*M. canettii*) show a greater genetic divergence yet are less virulent and less persistent.

**Objectives**

In order to better understand the mechanism underlying the smooth/rough variation and its implication in virulence, we are currently investigating smooth and rough morphotypes of *M. canettii* strains in more detail.

**Methods**

Stable smooth and rough morphotypes of different *M. canettii* strains were isolated after several passages on solid medium and were subjected to a whole genome sequence analysis by Illumina technology. Single-end short reads were aligned against the reference genome by using SHRiMP. Lipid analysis was done by chloroform/methanol/water extraction followed by thin layer chromatography.

**Conclusions**

Comparative genome analysis of the smooth and the rough morphotype revealed a difference in the *pks5* gene locus, which codes for a polyketide synthase that was found to be involved in lipooligosaccharide (LOS) synthesis in the fish pathogen *M. marinum*. More detailed analysis of this locus suggested the importance of *pks5* and a gene of the *pap* family (polyketide synthase associated protein) to be involved in

the phenomenon. Thus, the *pks5* locus seems to be involved in the smooth/rough variation in *M. canettii* through production of lipooligosaccharides, which could also play a role in the reduced virulence phenotype.

**FEMS-3147**  
**Mycobacteria**

**IDENTIFICATION OF NONTUBERCULOUS MYCOBACTERIA IN POTABLE AND RECYCLED WATER IN MEXICO CITY UNIVERSITY**

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**Background**

Nontuberculous mycobacteria (NTM) are ubiquitous microorganisms that can act as human pathogens in both, immunocompromised and immunocompetent individuals. Because until now it has not been described human-to-human transmission, the main source appears to be the exposure to water containing the microorganisms. All over the world an increasing number of NTM species with different profiles causing human disease represent a public health threat. However, a fast and reliable method to identify NTM in the water distribution system is lacking. The water distribution system and the recycled water for irrigation of Ciudad Universitaria campus of Universidad Nacional Autónoma de México (UNAM) were screened for NTM. We compared *hsp65* gene and 16S rRNA-23S rRNA (ITS) sequence in its ability to distinguish between NTM closely related species.

**Objectives**

The aim of this work was to compare the ITS and *hsp65* gene PCR-RFLP to identify NTM species isolated from recycled water and human use water in distribution systems of UNAM

**Methods**

56 recycled water samples and 163 potable water samples NTM's were grown in Lowenstein- Jensen medium and Middlebrook 7H10 medium. DNA from the isolated strains were identified by ITS and *hsp65* gene PCR-RFLP specific sequences. Amplifications were digested by HaeIII and CfoI for ITS and HaeIII and BstEII for *hsp65* gene. The electrophoresis gels of digested products were analysed with the Roth and Telenti algorithm.

**Conclusions**

Markers ITS and *hsp65* gene PCR-RFLP identified twelve species. Moreover the use of ITS complemented the *hsp65* restriction profiles. The sequences used are a good molecular tool for NTM species and subspecies identification.



**FEMS-1811**  
**Mycobacteria**

**THE ANALYSIS OF PEDIGREE AMONG MYCOBACTERIUM AVIUM SPP.  
PARATUBERCULOSIS INFECTED DAIRY HERD**

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**Background**

Johne's disease, chronic granulomatous enteric disease in dairy cattle, is caused by *Mycobacterium avium* spp. *paratuberculosis* (MAP) infection. The prevalence of the pathogen in US dairy farm is an approximately 68% and the pathogen induced \$200 to \$250 million economic loss in US dairy industry due to decreasing milk production and increasing cow replacement costs.

**Objectives**

Analysis of MAP positive pedigree for the evaluation of vertical MAP infection comparison with horizontal infection among dairy herd last 10years.

**Methods**

The serum MAP antibody detection enzyme-linked immunosorbent assay (ELISA) was applied for the identification of MAP infection among dairy herd and the test was performed biannually. The MAP positive or negative dam, offspring, sibling and offspring of sibling were recorded for 10 years (2005-2015).

**Conclusions**

The MAP positive or negative pedigree was constructed based on the ELISA test record and the association of each MAP positive individuals among dairy herd was not related with each other. The result from this study could suggest that the horizontal infection would be more considered MAP transmission rather than vertical infection among dairy herds, and it could provide a guideline of future MAP intervention program among dairy herds.

**FEMS-1566**  
**Mycobacteria**

**MOLECULAR DETECTION AND CHARACTERIZATION OF RESISTANT GENES  
IN MYCOBACTERIUM TUBERCULOSIS COMPLEX FROM DNA ISOLATED FROM  
TUBERCULOSIS PATIENTS IN THE EASTERN CAPE PROVINCE SOUTH  
AFRICA**

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**Background**

Tuberculosis (TB) in both animals and humans is caused by Mycobacterium tuberculosis complex

(MTBC) primarily transmitted by inhalation of aerosolized droplets containing the organism. Multi-drug resistance

(MDR) and extensive drug resistance (XDR) are evolutionary features of Mycobacterium tuberculosis to subvert the antibiotic regimes in place.

**Objectives**

The heavy burden of TB in South Africa motivated the investigation of MTBC prevalence among TB patients, amplification and sequencing of DNA amplicons known to confer resistance to TB drugs in Port Elizabeth.

**Methods**

Three thousand eight hundred and ten (3810) sputum specimens were processed and DNA isolated from the Eastern Cape Province, South Africa. DNA was amplified using the Seeplex® MTB Nested ACE detection assay. The agar-dilution proportion method was used to perform drug-sensitivity testing using 7H10 Middlebrook medium. Target genes known to confer resistance to first and second-line drugs were amplified and the amplicons sequenced.

**Conclusions**

One hundred and ninety (5%) DNA samples tested positive for MTBC and from the resistant profiles of the 190 positive samples, we noted that multidrug-resistant TB was identified in 189 (99.5%) with 190 (100%) patients infected with MTB resistant to isoniazid and 189 (99.5%) having MTB resistant to rifampicin. Other percentages of drug resistance observed including 40% pre-XDR and 60% of XDR. This study provides valuable data on the different kinds of mutations occurring at various target loci in resistant MTBC strains isolated from samples obtained from the Eastern Cape Province and reveal a high incidence of MDR amongst the positive samples from

Eastern Cape Province, South Africa.

**FEMS-0810**  
**Mycobacteria**

**MYCOBACTERIUM TUBERCULOSIS CELLWALL ASSOCIATED PROTEIN  
PROMOTES BACILLARY SURVIVAL BY INHIBITING OXIDATIVE STRESS AND  
AUTOPHAGY PATHWAYS IN MACROPHAGES AND ZEBRAFISH.**

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**Background**

*Mycobacterium tuberculosis* (*Mtb*) cellwall associated glycoproteins are involved in host pathgen interactions and pathogenesis.

**Objectives**

In our study, we identified mannosylated glycoproteins from purified *Mtb* cell wall. Our objective was to functionally elucidate the role of this protein in host immune responses and in pathogenesis.

**Methods**

*Mycobacterium tuberculosis* gene is cloned and expressed in *Mycobacterium smegmatis*. Orthologue of *Mtb* gene in *Mycobacterium marinum* was also deleted. Invasion assay was performed in HCT-116 and intracellular survival assay, ROS, autophagic and MAPK pathways were analyzed in infected macrophages.

**Conclusions**

The PRT activity measurement in *Mycobacterium smegmatis* expressing recombinant gene confirmed the mass spectrometry data. Using *Mycobacterium marinum*-zebrafish and the surrogate *MsmPRT* infection models, we proved that PRT is indeed involved in mycobacterial virulence. Histological and infection assays showed that *M. marinum* mutant was strongly attenuated in adult zebrafish and also survived less in macrophages; whereas infection with wild-type and the complemented PRT *M. marinum* strains showed prominent pathological features such as severe emaciation, skin lesions, hemorrhaging, greater weight loss and more zebrafish death. This intracellular survival reveals inhibition of autophagy, reactive oxygen species and reduced activities of superoxide dismutase and catalase enzymes in infected macrophages. Infection with *MsmPRT* also activated the MAPK pathway, NF- $\kappa$ B and inflammatory cytokines. In summary, we show that a novel mycobacterial mannosylated PRT acts both as a virulence and an immunomodulatory factor, suggesting that it may constitute a novel target for antimycobacterial drugs.



**FEMS-1779**  
**Mycobacteria**

**“TB-SPRINT ULTRA” : DEVELOPMENT OF A MICROBEAD-BASED METHOD FOR DETECTION OF MUTATIONS ASSOCIATED WITH SECOND-LINE DRUGS AND ETHAMBUTOL RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS ISOLATES**

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**Background**

New cost-effective, portable and personalized molecular methods to predict *Mycobacterium tuberculosis* drug-resistance are needed

**Objectives**

To up-grade 'TB-SPRINT', a previously developed assay that targets simultaneously CRISPR polymorphism and first-line drug resistance, to detect resistance to second-line drugs.

**Methods**

An assay targeting *gyrA* codon 94, *rrs* nucleotides 1401, 1402 and 1484, *eis* promoter -10, -12 and -14, and *embB* codon 306 was built on 16 reference DNA. The previous 59-Plex complexity was thus increased to a 75-Plex. To validate this assay a first set of 111 DNA samples from different geographical origin (Lithuania and Colombia) were blindly analyzed. Thirty-eight samples with pyrosequencing data allowed first estimates of performances.

**Conclusions**

Sensitivity, specificity and overall concordance rates of the first version of TB-SPRINT ultra in comparison with pyrosequencing were: 66.7% (6/9), 96.2% (25/26) and 88.6% (31/35), respectively, for FLQ; 96.2% (25/26), 100% (10/10) and 97.2% (35/36), respectively, for KAN; 90.9% (10/11), 100% (25/25) and 97.2% (35/36),

respectively, for AMK; 100% (2/2), 100% (35/35) and 100% (37/37), respectively, for CAP; and 100% (12/12), 100% (5/5) and 100% (17/17), respectively, for EMB. Altogether, 'TB-SPRINT ultra 1.0" was able to detect most prevalent mutations associated with second-line drug resistance with very good specificities on pure and concentrated samples. We are in the process of increasing sensitivity to broaden the applicability of this test. This work shows that tuberculosis resistance to first- and second-line drugs will soon be available as an 'all-in-one tube" assay. This assay will later be tested on biological samples directly.

**FEMS-2476**  
**Mycobacteria**

**INFECTIOUSNESS RATE IN PULMONARY TUBERCULOSIS PATIENTS WITH  
SMEAR POSITIVE OF SPUTUM IN KASHAN,IRAN**

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**Introduction**

Identifying time predictors of sputum smear conversion in patients with pulmonary tuberculosis (TB) could be used for planning and the counseling of TB patients. This study was conducted to assess the duration in which sputum smear was turned negative and end of infectiousness of smear positive in pulmonary tuberculosis patients in Kashan.

**Materials & Methods**

This was a cohort study was conducted on 50 smear positive Tb patients in Kashan from the year 2012 to 2013. Sputum smear was taken from pulmonary TB patients in Moslem TB Control Center. Sputum smear was done at the end of first, 2nd, 3rd, 4th and 8th week of treatment. A questionnaire including demographic information and clinical symptoms in addition to the result of HB and ESR were collected. Data analyzed was performed by SPSS 16.0.

**Results**

The result of analysis indicated that 23(46%) of the AFB smear positive tuberculosis were male and 27(54%) were female. The mean age of the patients was  $54.7 \pm 22.7$  years, while 21(42%) were under the age 49 and 29(58%) were over the age 49 years. The result also showed that 20 (40%) patients were Iranian and 30(60%) was from Afghanistan. The most frequently observed cases (90%) were from urban areas and 68% of the cases were outpatient. The highest frequency of clinical symptoms were fever 36 (72%) and dyspnea 21(42%), respectively. The negative result of sputum smear at the end of 2th week was observed in 8(16%), 3th week in 20 (40%), 4th week in 23(46%) and 8th week in 31(62%) patients, respectively. There was positive smear in 19(38%) patients. There was no significant association between the age, sex, nationality and positive result of smear at the second month of treatment. The result of ANOVA revealed that there was a significant effect of time on the infectiousness.

**Conclusion**

Considering the high rate of smear positive AFB at the end of second month of treatment, assessment of Dots quality and the incident of drug resistance among mycobacterium tuberculosis in Kashan is necessary and important.

**FEMS-2623**  
**Mycobacteria**

## **MULTIPLE COLD ABSCESSSES DUE TO MYCOBACTERIUM TUBERCULOSIS**

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### **Background**

The musculoskeletal system involvement of tuberculosis (TB) other than spinal TB is a rare and less-known entity. Furthermore symptoms of musculoskeletal involvement of TB infection are non-specific, relatively slow and indolent

### **Objectives**

We report a 55-year-old patient suffering multiple cold abscesses in adjacent soft tissues; emphasising significant clinical and imaging findings

### **Methods**

### ***Case presentation:***

A 55 year old man from Kashan-Iran presented with a 4×4 cm mass without discharge and pain in left buttock from 9 months ago treatment with cephalexin was not successful and other masses appeared in anterior of left leg, left clavicle and under right 12 th rib and right scapula. other complains were 30 kg weight loss, anorexia, anemia and occasional night fever. he was cachectic and pale and afebrile.

there was no tenderness and discharge in all masses. in sonography, collection having debris and gas extending from bone to soft tissue was reported. Mass of elbow was aspirated and acid fast bacilli was seen in smear of aspirated sample. anti TB treatment was started and after 6 months he was well and all masses were disappeared

### **Conclusions**

TB must be considered in differential diagnosis of multiple cold abscesses even in an immunocompetent patient and treatment can be effective even in advanced stage.

**FEMS-1540**  
**Mycobacteria**

## **MOLECULAR TYPING OF MULTIDRUG-RESISTANT AND DRUG SUSCEPTIBLE MYCOBACTERIUM TUBERCULOSIS IN ASIAN COUNTRIES**

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### **Background**

Emergence and spread of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) is a serious problem. The treatment needs significantly longer period than in drug susceptible TB and the outcome tends to be disappointing.

### **Objectives**

To understand the mechanism of the development of MDR-TB, we should have information about the genetic background of each *M. tuberculosis* strain. In current study, we collected TB isolates in three Asian countries, Nepal, Bangladesh and Japan, and compared the genetic characteristics between MDR-TB and drug susceptible TB groups.

### **Methods**

TB isolates were collected in each country as follows and the spoligotype was determined, Nepal: 154 MDR-TB and 95 susceptible, Bangladesh: 182 MDR-TB and 50 susceptible, Japan: 88 MDR-TB and 29 susceptible. In MDR-TB, drug resistance determining regions of *rpoB*, *katG* and *inhA* were also analyzed.

### **Conclusions**

In current study, we found some of the spoligotype lineages increased its percentage among MDR-TB, e.g. Beijing type and T1 (SIT53), which seemed to suggest their clonal expansion. Those isolates usually possessed a so-called low-fitness-cost mutation, *katG* G944C, and they might have additional advantageous characteristics.

Further investigation seems to be needed to elucidate the mechanism of the emergence of transmissible MDR-TB to prevent the spread of the lethal pathogen.

**FEMS-2778**  
**Mycobacteria**

**HIGH DETECTION OF ATYPICAL MYCOBACTERIOSIS IN OAXACA, MEXICO**

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**Background**

The reemergence of tuberculosis worldwide has forced efforts to control infections caused by mycobacteria, developing tools to assist in the diagnosis of early, quickly and efficiently, with the aim of reducing morbidity related to Mycobacteria.

**Objectives**

The aim of this work was to detect and differentiate between an infection caused by *Mycobacterium tuberculosis* complex (MTBC) and atypical mycobacteria (NTM) in patients with clinically suspected pulmonary tuberculosis.

**Methods**

Multiplex PCR for detection of CMTB and NTM was assessed; 120 sputum samples were collect from patients with suspected TB, which were divided for PCR analysis and culture.

**Conclusions**

In 39.2% of the samples, mycobacterial DNA was detected; by culture, only 12.5% were positive. These results highlight the importance of the implementation of molecular techniques in the detection of mycobacterial infection with clinical correspondence. In the samples we detect MTBC (38%), *M. avium* (4.2%), *M. intracellulare* (4.2%); *M. indicus* (2.1%), and the remaining 51.5% identification was not possible since only the 16S rRNA and rpoB genes were detected, representing a high percentage of infection NTM, most of these samples were not possible to cultured. In previous years it has been detect by culturing *M. fortuitum* infection, the next step is to design specific primers for the precise identification of the NTM that represent a high percentage of infection in the population of Oaxaca.

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**FEMS-1338**  
**Mycobacteria**

**RAPID DIAGNOSIS OF MYCOBACTERIUM TUBERCULOSIS WITH TRUENAT  
MTB: A NEAR CARE APPROACH**

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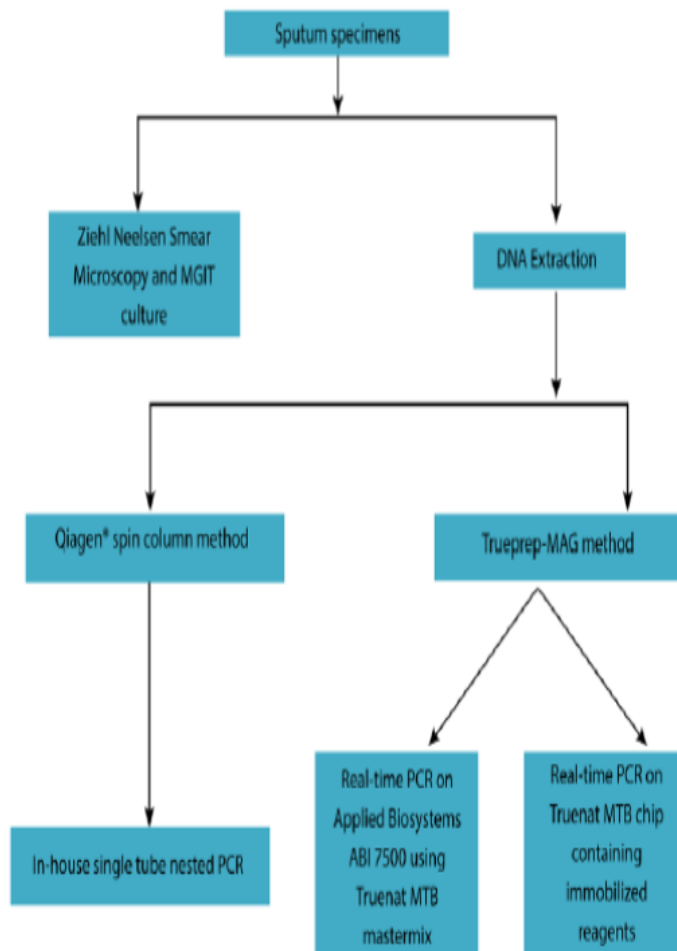
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**Background**

Control of the global Tuberculosis (TB) burden is hindered by the lack of a simple and effective diagnostic test that can be utilized in resource-limited settings. There has been considerable interest in the miniaturization of the PCR platform as this would confer advantages such as reduction in cost of instruments and tests, faster turnaround times and enhancement in the availability and accessibility of PCR tests in resource-poor geographies. With the combined advantages of affordability, simplicity in operations, diagnostic sensitivity and portability, micro-PCR devices are strong candidates for widescale use among the peripheral laboratories of India

**Objectives**

Objectives are as follows:



## Methods

We evaluated the performance of Truenat MTBTM, a chip-based nucleic acid amplification test in the detection of Mycobacterium tuberculosis (MTB) in clinical sputum specimens from 226 patients with suspected pulmonary tuberculosis (TB). The test involved sputum processing using Trueprep-MAGTM (nanoparticle-based protocol run on a battery-operated device) and real-time PCR performed on the Truelab UnoTM analyzer (handheld, battery-operated thermal cycler). Specimens were also examined for presence of MTB using smear microscopy, liquid culture and an in-house nested PCR protocol. Results were assessed in comparison to a composite reference standard (CRS) consisting of smear and culture results, clinical treatment and follow-up, and radiology findings.

## Conclusions

This preliminary study shows that the Truenat MTB test allows detection of TB in approximately one hour and can be utilized in near-care settings to provide quick and accurate diagnosis.

**FEMS-1094**  
**Mycobacteria**

## **EFFECTS OF PROBIOTICS-INDUCED MACROPHAGE POLARIZATION ON INTRACELLULAR KILLING IN MYCOBACTERIUM TUBERCULOSIS**

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### **Background**

Isoniazid (INH) is known to have a bactericidal action against actively replicating *M. tuberculosis* (Mtb). However, it has little effects against slowly replicating bacilli. Mtb reverts from nongrowing bacilli in M1 macrophages to growing bacilli in M2 macrophages and can be susceptible to INH. Therefore, the modification of host immune system is crucial for more effective elimination of persisters in non-replicating state.

### **Objectives**

Therefore, the modification of host immune system is crucial for more effective elimination of persisters in non-replicating state.

### **Methods**

Mouse splenocytes were cultured for 5 days and non-adherent cells were removed. The fermented soybean paste isolated *Bacillus lentus* (BL) was applied to cells for M1 and *Lactobacillus brevis* (LB) isolated from Kimchi, the fermented vegetable, for M2 with 48 hours of infection period. LPS for M1 and IL-4 for M2 were used as a positive control and were run in parallel with every assay. Activated cells were infected with *M. tuberculosis* H37Rv and INH was added 3 days after infection. After 4 days of incubation, number of intracellular Mtb was determined at 14-16 days of infection.

### **Conclusions**

BL had been shown to induce M1. However, LB induced high levels of IL-10 and arginase-1 secretion which is characteristics of M2 macrophage polarization. LB was shown to induce M2 allowing the replication of mycobacteria that increased the efficacy of INH. It could be concluded that the combined use of anti-tuberculosis chemotherapy and immunotherapeutic microbiology might be more effective for eradication of persisters Mtb in macrophages.

**FEMS-0757**  
**Mycobacteria**

## **IDENTIFICATION OF A PUTATIVE TETR REGULATOR IN MYCOBACTERIUM SMEGMATIS**

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### **Background**

*Mycobacterium tuberculosis* (MTB) is able to survive under physical chemical insults, such as nitrosative and acid stress, within the macrophage host niche. By gene expression studies under stress conditions in the model organism *Mycobacterium smegmatis* mc<sup>2</sup>155 we show the down regulation of the *MSMEG\_3765* gene, whose deduced amino acid sequence shares 74% identity with *Mycobacterium tuberculosis* H37Rv *Rv1685c* gene product. Both genes are defined as members of TetR transcriptional regulators, often serving as repressors.

### **Objectives**

Role of the *MSMEG\_3765* gene in stress response. Isolation and characterization of a *M.smegmatis* mutant strain, carrying a null mutation in *MSMEG\_3765*.

### **Methods**

Transcriptional analysis of the *MSMEG\_3765* locus was performed by RT-PCR; the *MSMEG\_3765* gene expression was evaluated by Real Time PCR after acid-nitrosative multi-stress.

The mutant strain was constructed by a two step homologous recombination process. The upstream and downstream regions of *MSMEG\_3765* (1-Kb flanking region) were cloned into a pNIL series vector together with marker genes obtained from the pGOAL series, leading to a suicide delivery system. The pFP1 recombinant vector was used to transform *M.smegmatis* competent cells, and the *M.smegmatis*  $\Delta 3765$  mutant strain was isolated.

### **Conclusions**

The *MSMEG\_3765* was shown to be the last of a four genes operon. Real Time PCR analysis revealed that *MSMEG\_3765* is four times less expressed under acid-nitrosative multi-stress than standard growth conditions. To elucidate the role of *MSMEG\_3765* gene under stress conditions, stress survival and proteome analyses of mutant strain are in progress.

**FEMS-1805**  
**Mycobacteria**

**INCIDENCE OF PARATUBERCULOSIS IN WILD RUMINANTS IN BULGARIA -  
FIRST VETERINARY STUDY WITH IMPLEMENTED COMPLEX DIAGNOSTIC  
TECHNIQUES**

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Background: Paratuberculosis is one of the most important diseases in cattle and wild animals and with great importance to the international trade market. That is why the application of combination of rapid methods will allow developing a methodology for control of paratuberculosis in Bulgaria, to conduct epidemiological analysis and to assess the risk to animals and humans.

Objectives Application of pathomorphological, microbiological and molecular methods for detection and identification of *Mycobacterium avium* subsp. paratuberculosis (MAP) in tissue samples.

Methods: Study samples included 50 diagnostic materials (mesenteric lymph nodes, small intestine) from deers and mouflons in Bulgaria, suspected for paratuberculosis in the period of 2009-2013. Bacterial cultivation was carried out by using Middlebrook 7H9, Herrolds and Löwenstein-Jensen media. For molecular methods was used MAP Vet kit (Sacace Biotechnologies, Italy).

Results and Conclusions: Typical for MAP pathomorphological lesions were observed in 4 mouflons and 3 deers. MAP isolates grow very well in the Middlebrook 7H9 and Herrold media but did not grow on the Löwenstein-Jensen medium even after 6 months cultivation. The presence of MAP was confirmed by PCR methods. In conclusion, we present here the first veterinary data for diagnostics of paratuberculosis in wild animals in Bulgaria. Development of this scientific field will deepen the knowledge of the properties and early detection of MAP, its significance for infectious pathology, prevention and control and will add new information to the global database for distribution of paratuberculosis in Bulgaria.

**FEMS-1302**  
**Mycobacteria**

**THE PEPTIDOGLYCAN REMODELLING ENZYME RPF<sub>B</sub> OF MYCOBACTERIUM TUBERCULOSIS IS TRANSCRIPTIONALLY REGULATED BY MTR<sub>A</sub> AND SIGMA FACTOR B.**

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**Background**

*Mycobacterium tuberculosis*, the causative pathogen of tuberculosis, is a global burden to human health. With the emergence of multi drug resistance, extensive drug resistance and co-infection with HIV, tuberculosis kills 1.7 million people every year. *Mycobacterium tuberculosis* lies dormant within its host over a long time, emerging from dormancy only when conditions are favorable. Both dormancy and reactivation represent processes which are likely to be good therapeutic targets.

**Objectives**

The resuscitation promoting factors are hydrolytic enzymes, which are required for resuscitation of dormant mycobacteria. Resuscitation promoting factor B (RpfB), a peptidoglycan remodelling enzyme is required for reactivation of mycobacterium from chronic infection *in vivo*, underscoring the need to understand its transcriptional regulation.

**Methods**

Transcriptional regulation of *rpfB* has been analyzed using promoter GFP- fusions and in vitro transcription assays. Transcription start point was determined by 5'-RACE. In vitro transcription and EMSA confirmed the recognition of the *rpfB* promoter by sigma factor, SigB and transcription factor, MtrA respectively. Streptavidin pull down assay with a biotinylated DNA fragment and chromatin immunoprecipitation confirmed association of MtrA with the *rpfB* promoter region. Binding site of MtrA on the *rpfB* promoter was identified by DNaseI footprinting assay.

## Conclusions

SigB and MtrA are the two regulators of *rpfB*. MtrAB coordinates cell wall homeostasis. A likely role in cell wall metabolism is strengthened by this report that MtrA regulates expression of the peptidoglycan hydrolase, RpfB. The insights into regulation of *rpfB* are therefore relevant in our quest to uncover new bacterial targets for drug development.

**ROLE OF THE N-TERMINUS OF THE MYCOBACTERIAL MEMBRANE PROTEIN MCTB IN THE LOCALIZATION, STRUCTURE AND RESISTANCE TO COPPER.**

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**Background**

We previously described the protein Rv1698 of *Mycobacterium tuberculosis* as involved in an unknown mechanism of resistance to toxic copper ions: mutants of *M. tuberculosis* and *M. smegmatis* lacking functional expression of the gene accumulated copper while wild-type cells did not. Rv1698 is a cell envelope, channel-forming protein and we hypothesized it could be part of a copper efflux machinery. We named it MctB (Mycobacterial copper transport protein B).

**Objectives**

To gain insight on the contribution of MctB to copper resistance, we aimed to characterize its structure.

**Methods**

We solved the structure of recombinant MctB lacking its putative signal peptide by X-ray crystallography. We determined the composition of the mature protein by mass spectrometry analysis. By expressing translational fusions of *mctB* to various reporter genes within phenotypic assays, we investigated the role of the N-terminal hydrophobic  $\alpha$ -helix on the localization and function of the protein. We analyzed the oligomeric state of MctB by size-exclusion chromatography and electron microscopy (EM).

**Conclusions**

In contrast with the established biophysical properties, the crystal structure of recombinant MctB did not show characteristics of a classical channel protein, namely the presence of a channel or contiguous hydrophobic surfaces. We showed that the N-terminus – thought to be a classical signal peptide – (i) is still present in the mature form of MctB, (ii) is required for the translocation and activity of MctB outside the cytosol and (iii) plays a critical role in its oligomerization. Finally, we propose an oligomeric model of MctB based on EM data that helps explain MctB properties.



**FEMS-1674**  
**Mycobacteria**

## **MOLECULAR CHARACTERIZATION OF MYCOBACTERIUM ORYGIS ISOLATES FROM WILD ANIMALS OF NEPAL**

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### **Background**

*Mycobacterium tuberculosis* complex (MTC) species cause tuberculosis (TB) in humans and animals. Recently, we isolated acid fast bacilli from a spotted deer (*Axis axis*) and a blue bull (*Boselaphus tragocamelus*) in Nepal. They showed atypical colony characteristics.

### **Objectives**

Genotypic characterization of bacterial isolates helps to understand the nature of bacteria. In this study, we aimed to characterize the bacilli isolated from the spotted deer and the blue bull in detail.

### **Methods**

TB suspected lesions from the lung and lymph node in the deer and the lung in the blue bull were collected during postmortem and cultured on Löwenstein-Jensen media. Spoligotyping, multi-locus variable number of tandem repeats analysis (MLVA), region of difference analysis (cfp32, RD9 and RD12) and SNP typing were performed to identify species and to get molecular epidemiological insights.

### **Conclusions**

Isolates from both animals showed a spoligotype SIT587 in SITVIT WEB database as of January 2015. RD analysis results (cfp32: +, RD9 - and RD12-) and SNP data in *gyrB*, *mmpL6*, *TbD1*, *PPE55* and *Rv2042c* identified these isolates to be *M. orygis*. In addition, MLVA of these isolates showed a similar pattern with many other reported *M. orygis* isolates. *M. orygis* have been isolated from many animals and humans of South Asian countries. Our finding may indicate *M. orygis* to be endemic in the sub-continent. Additionally, the identification of a new member of MTC in Nepal may suggest the complexity of tuberculosis in the country. Further investigation with increased sample size and various host animals including human will help to understand the ecology and epidemiology of *M. orygis*.

**FEMS-2931**  
**Mycobacteria**

**MIXED INFECTION IN TUBERCULOSIS: THE CRUCIAL PHENOMENA IN  
OUTCOME OF TREATMENT**

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**Background**

*Mycobacterium tuberculosis* (MTB) is among the most successful human pathogenic bacteria worldwide and is responsible for extensive morbidity and mortality, with approximately 2 million deaths each year thought to be due to primary infection, reactivation of latent infection, or re-infection with a new strain. Molecular fingerprinting has differentiated the MTB strains. Most of these studies have shown that each patient is infected with one strain. However, by using more sensitive PCR based technique such as MIRU-VNTR, it is possible to determine co infection with multiple strains of MTB.

**Objectives**

The aim of the current study was to determine the prevalence of mixed infection and clonal complexity in Iranian patients by MIRU-VNTR technique. Additionally, we aimed to determine whether mixed infection results in differences in clinical manifestation or response to treatment.

**Methods**

The PCR was designed to amplify a standard set of 24 MIRU-VNTR loci from genomic DNA extracted using primers described by Supply et al. PCR products were electrophoretically separated on 1.8% agarose gels. In addition, the presence of double alleles was confirmed by two independent rounds of PCRs

**Conclusions**

The MIRU-VNTR method was able to identify mixed infection in at least 25% (17/67) of the cases. Mixed infections occur at high prevalence among Iranian TB patients. The presence of multiple strain infections had high impact on the clinical manifestation and response to anti tuberculosis treatment for individual patients.

**FEMS-2052**  
**Mycobacteria**

**PRESENCE OF MUTATIONS IN GENES ASSOCIATED WITH ANTIBIOTIC RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS STRAINS FROM MICHOACÁN, MEXICO**

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**Background**

The six-month treatment against tuberculosis involves rifampin, isoniazid, pyrazinamide, ethambutol and streptomycin. Associations between mutations in specific genes (*rpoB* to rifampicin; *katG* and promoter *mabA* to isoniazid; *pncA* to pyrazinamide; *embB* to ethambutol; *rrs* and *rpsL* to streptomycin) and resistance to antibiotics have been reported. The frequency of these mutations varies in each country and region within a country, so in order to design diagnostics tools, the relationship between mutations and first line resistance patterns in each country must be known.

**Objectives**

Identify mutations associated to antibiotic resistance in strains of *M. tuberculosis* from patients in the state of Michoacán, Mexico.

**Methods**

Thirty-three strains of *Mycobacterium tuberculosis* were analyzed. The susceptibility patterns were determined using BACTEC-MGIT. Mutations were detected by PCR amplification, sequencing, and comparison with the TB Drug Resistance Mutation Database.

**Conclusions**

21.2% of the studied strains were resistant to rifampin. The genes *katG* and *rpoB* showed the highest mutation percentage (30.3% and 27.2%, respectively). 54.5% of the strains presented at least one mutation in any of the analyzed genes. Our analysis of resistant strains found both new and previously reported mutations such

as the *katG* Ser315Thr. The four analyzed strains isolated from patients with meningeal tuberculosis showed extensive drug susceptibility of first line antibiotics. Only two of the latter strains had mutations in the *katG* gene: strain 049 with synonymous Ala411Ala mutation having no influence on antibiotics sensitivity, and 040 with Arg463Leu mutation reported as producing resistance in some cases. 62.5% of the strains resistant to drugs showed some resistance mutation.

**VARIATIONS IN THE SEQUENCES OF VIRULENCE GENES HARBORED BY MYCOBACTERIUM TUBERCULOSIS STRAINS FROM MICHOACAN**

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**Background**

The *Mycobacterium tuberculosis* genome contains two large families of genes called *pe* and *ppe*, some of which encode proteins involve in virulence and the evasion of the immune response. These genes are highly polymorphic and these polymorphisms have been associated with the pathogenicity of strains that are presented.

**Objectives**

To find relationships between polymorphisms in *pe/ppe* gene families and certain genotypes.

**Methods**

59 *M. tuberculosis* strains obtained of tuberculosis patients from Michoacán, México, were analyzed. The species, lineage, family and genotype of the strains were previously determined. The *pe\_pgrs18* and *pe\_pgrs26* genes were amplified and sequenced. Polymorphisms were found by alignment with ClustalX and MEGA6 software against homologous sequences retrieved from GenBank.

**Conclusions**

The *pe\_pgrs18* gene sequences from strains belonging to T, LAM and some not previously reported (NPR) families from the Euro American lineage, EAI strains from Indo Oceanic lineage and Beijing strains from East Asia lineage, harbor SNPs in positions 54-266. Sequences from the same gene, but from Harlem, X and different NPR strains from Euro American lineage, NPR strains from Indo Oceanic lineage and Ghana strains from West Africa lineage, showed SNPs and insertions in positions 295-510. The *pe\_pgrs26* gene harbor SNPs, insertions and deletions; X and T strains shared the same SNPs, while EAI strains showed their own set of shared SNPs. Some detected polymorphisms are consistent with previous reports, while others are not. The gene *pe\_pgrs18* was highly polymorphic. There is a relationship between genotypes and certain polymorphisms in *pe* and *ppe* families.

**FEMS-2492**  
**Mycobacteria**

**GENE EXPRESSION PROFILE OF BIOMARKER CANDIDATES IN  
MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS INFECTED CATTLE**

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**Background**

Johne's disease(JD) is a chronic disease of ruminants caused by *Mycobacterium avium* subsp. *paratuberculosis* (MAP). JD has very long incubation periods, resulting in difficulties in diagnosing the disease, especially regarding the fecal shedders of MAP without any clinical signs. Current MAP diagnosis techniques lack sensitivity in the detection of early stage of MAP infection. Therefore, attempts were made to findout biomarker to detect early responses to MAP infection.

**Objectives**

To develop enhanced diagnosis of early stage of MAP infection, biomarkers were evaluated by analysis of immune-related genes in MAP-infected cattle.

**Methods**

Total RNA was extracted from whole blood of 32 cattle characterized by ELISA and IS900/IS-MAP PCR. Expression of 24 genes was analyzed by real-time PCR to validate the biomarker candidates. The expression level was determined by the 2<sup>- $\Delta\Delta$ Ct</sup> method using  $\beta$ -actin, as a reference. The expression level was compared to the control group to determine the expression-fold change of each gene.

**Conclusions**

Based on the present study, four biomarker candidates showing significant changes were proposed as potential biomarkers of JD. CCL4 and CXCR3 expression were significantly decreased in group 1. On the contrary, CXCL9 expression was significantly decreased in group 2. KLRB1 expression was significantly increased in group 2. These biomarkers might be used for development of enhanced diagnostic tool of early stage of MAP infection in further study. This work was carried out by the grant from RDA, Korea(Project No. PJ008970012015)

**FEMS-2496**  
**Mycobacteria**

**EVALUATION OF MYCOBACTERIUM AVIUM SUBSP. PARATUBERCULOSIS ANTIGENS TO DETECT SUBCLINICAL STAGE OF INFECTION IN CATTLE**

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**Background**

Johne's disease, caused by *Mycobacterium avium* subsp. *paratuberculosis*(MAP) is one of the most widespread and economically important disease in cattle. Current diagnostic methods are based on detection of MAP antibodies in milk or serum samples or isolation of causative agent. However, these diagnostic techniques are often not applicable in subclinical infection because of relatively low sensitivity. Therefore, finding new antigen candidates highly react with host immune system has been required.

**Objectives**

To detect subclinical stage of infection effectively, several antigen candidates were selected from previous reports. Selected antigen candidates were analyzed about their efficiency.

**Methods**

Complete gene sequences of antigen candidates were cloned and expressed by cold shock expression system with pCold™ I vectors and One shot® chemically competent *E. coli* Top10 cells. Recombinant proteins were tagged by 6-histidine and purified by His SpinTrap™. Serological samples were prepared from cows with different stages of the disease divided into four groups according to stage of infection. Reactivity with these four groups of sera and each antigen was evaluated by western blot.

**Conclusions**

Total of 9 antigen candidates were selected(MAP0862, MAP3817c, MAP2077c, MAP0860c, MAP3954, MAP3155c, MAP1204, MAP1087, MAP2963c) which have MAP specific and/or have high immune responsibility to infected animals. Using four groups of serological samples, it could be evaluated whether the antigen possibly

detect subclinical infection or not. Possible antigen cocktail will be used for development of sensitive ELISA. This work was carried out with Project No. PJ008970012015.



**COMPLEX INFECTION AS A POTENTIAL MECHANISM FOR HETERO-RESISTANCE AND RIFAMPIN RESISTANCE IN MYCOBACTERIUM TUBERCULOSIS**

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**Background**

Rifampin is a key component of the first-line therapy against *Mycobacterium tuberculosis*. Rifampin resistance was reported to be caused by mutation in *rpoB* gene, while 5% of the resistant strains showed no mutation. Hetero-resistance is considered as the preliminary stage to the full resistance in *M. tuberculosis*.

**Objectives**

This study investigated the possible mechanisms conferring hetero-resistance and rifampin resistance without *rpoB* gene mutations.

**Methods**

Two hundred and twelve clinical isolates were subjected to *rpoB* gene sequencing to examine the occurrences of *rpoB* mutations and hetero-resistance. The standard 24-locus variable-number tandem repeat (VNTR) method was used to identify complex infection, which consisted of mixed infection (the presence of strains with different VNTR patterns at  $\geq 2$  loci in one sample) and clonal heterogeneity (different VNTR patterns at a single locus).

**Conclusions**

We found that 3.8% of isolates ( $n = 8$ ) had rifampin resistance of no mutation in *rpoB* gene, in three among which dual peaks from the sequencing results were revealed and therefore identified as rifampin hetero-resistant. The main peak was wild type and the secondary one was mutated (Cys-526, Leu-531 and Tyr-526). All three hetero-resistant isolates were caused by complex infection (18, 8.5%), which included mixed infection (7, 3.3%) and clonal heterogeneity (11, 5.2%). Rifampin resistance with no mutation in *rpoB* gene was significantly associated with complex infection ( $\chi^2$ , 31.21,  $P < 0.01$ ), especially mixed infection ( $\chi^2$ , 56.78,  $P < 0.01$ ). Accordingly, it is necessary to examine complex infection, a possible mechanism for hetero-resistance and rifampin resistance without *rpoB* gene mutations.

**FEMS-0545**

**Microbial cell biology and structure**

## **CYTOXIC AND ANTIMICROBIAL POTENTIAL OF PREMNA RESINOSA**

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### **Background**

Herbal drugs have been used since ancient times as remedies for the treatment of a variety of ailments. *Premna* is a member of family Verbenaceae. Some of *Premna* species are known to exhibit cytotoxic, antimicrobial, antioxidant, antipyretic, hypoglycemic and cardiogenic activities.

### **Objectives**

In this study *P. resinosa* methanolic extract as well as its constituents were evaluated for antimicrobial and cytotoxic activity.

### **Methods**

*Premna resinosa* powdered plant was extracted with methanol and constituents were isolated by different chromatographic techniques and identified by spectroscopic methods. The extracts and compounds were tested for antimicrobial *in vitro* against *Staphylococcus aureus*, *Bacillus subtilis*, *Acinetobacter baumannii*, *E. coli*, *Salmonella typhimurium*, *Shigella flexneri* and *Enterococcus faecalis*. In addition, they were tested against human medulloblastoma (Daoy), human hepatocellular carcinoma (HepG2) and human melanoma (SK-Mel-28).

### **Conclusions**

Seven constituents were identified from *P. resinosa*. They are Quercetin (1), 3-Methoxyquercetin (2), Kaempferol (3), 3-Methoxykaempferol (4), Myricetin 3,7,3'-trimethyl ether (5), Lupeol (6), Stigmasterol (7). The dichloromethane extract exhibited the highest antimicrobial effect, meanwhile, Noticeable cytotoxic activity was shown by the crude methanolic extract against Daoy cell line. The antimicrobial activity of the isolated compounds showed higher sensitivity of Gram-positive bacterial strains than the Gram-negative.

**FEMS-3094**

**Microbial cell biology and structure**

**NEW INSIGHTS IN THE BUTYRATE PHOTOASSIMILATION IN  
RHODOSPIRILLUM RUBRUM S1H**

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**Background**

Purple non-sulfur bacteria display a great metabolic versatility and are able to assimilate volatile fatty acids (VFAs) through photoheterotrophic metabolism. One of these bacteria, Rhodospirillum rubrum S1H, was subsequently selected by the European Space Agency for its ability to metabolize VFAs and to remove them in its bioregenerative life support system.

**Objectives**

Here we aim to offer a better view of the still misunderstood photoheterotrophic metabolism of butyrate.

**Methods**

A global understanding of butyrate photoassimilation in purple non-sulfur bacteria was obtained through a multidisciplinary approach including quantitative SWATH-MS proteomic analysis, activity assays, bacterial growth analysis and VFAs consumption monitoring.

**Conclusions**

Butyrate is partially converted into acetyl-coA. Because R. rubrum is an isocitrate lyase lacking organism, an alternative anaplerotic pathway to glyoxylate cycle should be used to replenish the TCA cycle with intermediates for biosynthesis. We suggest here that butyrate could be converted into propionyl-CoA and succinate through ethylmalonyl-CoA pathway and valine degradation pathway.

We also observed that butyrate assimilation occurs only if carbonate is supplied. In accordance with the observed upregulation of RuBisCO, CO<sub>2</sub> fixation is most probably used as redox balancing reaction necessary to sustain butyrate assimilation.

Finally, we showed an inhibition of the butyrate assimilation by acetate when they are provided together at an equivalent net carbon concentration. This effect could be due

to the observed downregulation of key enzymes of the butyrate assimilation pathway when acetate is used as carbon source.

Altogether our data allows better understanding of metabolic pathway involved in butyrate photoassimilation in *R. rubrum*.

**FEMS-2328**

**Microbial cell biology and structure**

## **THE UNIQUE SHEATHS OF METHANOGENIC ARCHAEA ARE FUNCTIONAL AMYLOID STRUCTURES**

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### **Background**

Amyloids are highly organized protein or peptide polymers characterized by a cross- $\beta$ -sheet structure. These protein structures were for many years solely considered an abomination associated with incorrect protein folding, leading to devastating neurodegenerative diseases, such as Alzheimer's disease. However, within the last decade it became clear that the amyloid protein fold has many beneficial roles in nature. In bacteria they are used as adhesive fimbriae and for the coating of spores. Fungi use them to modulate the hydrophobicity of aerial hyphae and spores and in the form of prions as a means of phenotype regulation. In mammals, amyloids are used as a scaffold for the skin pigmentation and as a storage form for hormones. However, functional amyloids have never been shown within the Archaea domain of life.

### **Objectives**

Members of the archaeal genera *Methanospirillum* and *Methanosaeta* grow as filamentous chains of rod-shaped cells within tubular paracrystalline sheaths. These sheaths share many properties with the functional amyloids. They are proteinaceous and display high stability toward chemical and thermal denaturation and display a cross- $\beta$ -sheet structure. Accordingly, we hypothesized that the archaeal sheaths could represent a novel type of functional amyloid.

### **Methods**

We employed amyloid-specific conformation antibodies and biophysical techniques to confirm the amyloid nature of the sheaths.

### **Conclusions**

Our work represents the first report of a functional amyloid from the archaeal domain of life. The amyloid nature explains the extreme resistance of the sheath, the elastic properties that allow penetration of diffusible substrates, and how the sheaths are able to split and elongate outside the cell.

## FEMS-1301

### Microbial cell biology and structure

#### EXTRACELLULAR STRUCTURES OF PELAGIC FORMOSA

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#### Background

*Flavobacteria* contribute to the degradation of complex organic matter, in particular proteins and polysaccharides. During the decay of a spring phytoplankton bloom in the German Bight of the North Sea near Helgoland in 2009 a successive occurrence of different *Flavobacteria* clades represented up to 60% of the bacterial community (Teeling, H. *et al.*, 2012). Strains of the genus *Formosa* in the marine clade of *Flavobacteriaceae* were recently isolated from this site (Hahnke, R.L. *et al.*, 2014). Large extracellular structures were detected (Hahnke, R.L. *et al.*, 2014), however their function is still not understood.

#### Objectives

We started to study such appendages to gain new insights into their biological role within the pelagic bacterioplankton.

#### Methods

Light microscopy, transmission electron microscopy (TEM) and scanning electron microscopy (SEM) showed at cells of *Formosa* clade A segmented chain-like appendages with a width between 40 to 100 nm. A first membrane preparation by cells disintegrated by pressure change and by differential centrifugation revealed TonB-dependent receptors, an OmpA protein, one SusD-like protein and two hypothetical proteins in the outer membrane enriched fraction. Similarities in the protein pattern were observed for the other pelagic strain, *Formosa* clade B. In contrast, *Formosa agariphila* isolated from green algae revealed a different protein pattern.

#### Conclusions

In this contribution, we will report the outer membrane/appendage proteome as prepared by different protocols. In addition, the appearance of appendages as

response to the physiological situation of the cells may provide first insight into the ecological importance of these appendages.

**FEMS-1619**

**Microbial cell biology and structure**

**INVESTIGATION OF THE SIGNAL LEADING TO SURFACE LOCALIZATION OF LIPOPROTEINS IN CAPNOCYTOPHAGA CANIMORSUS**

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**Background**

The gram negative *Bacteroidetes* are prominent members of the human intestinal flora, where they are responsible for the breakdown of all major classes of host and dietary glycans. This ability is encoded by so-called Polysaccharide Utilization Loci (PUL), of which the starch utilization system (Sus) of *B. thetaiotaomicron* is the archetype. The Sus is a cell envelope-associated multiprotein complex characterized by the coordinated action of several proteins and lipoproteins involved in starch binding, degradation and internalization into the periplasm. *Bacteroidetes* genome sequencing projects showed that PUL are a hallmark of the phylum. PUL-encoded lipoproteins are surface exposed and little is known on how these proteins are recognized and targeted to the cell envelope.

**Objectives**

We aimed at understanding how the bacterial cell distinguishes between periplasmic and surface lipoproteins.

**Methods**

We used as model organism *Capnocytophaga canimorsus*, an oral commensal of dogs and cats, which can cause severe human infections. An *in silico* approach allowed the identification of a putative signal region at the N-terminus of the lipoproteins and we therefore performed a mutagenic screen on this region on the PUL-encoded surface mucinase Ccan\_17430. The mucinase surface localization of the N-terminal mutants was detected by monitoring mucin degradation.

**Conclusions**

We found a region needed for protein stability, preventing proteolytic cleavage and/or miss-localization of the protein. Further work is needed to characterize the signal and to determine whether the protein degradation observed for several mutants is due to protein instability or is a consequence of miss-localization.



**FEMS-2983**

**Microbial cell biology and structure**

**THE STACK: A NEW BACTERIAL STRUCTURE ANALYZED IN THE ANTARCTIC BACTERIUM *PSEUDOMONAS DECEPTIONENSIS* M1T BY TEM CRYO-TEM AND TOMOGRAPHY**

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**Background**

Improvements in TEM and Cryo-TEM techniques and the use of Cryo-electron tomography (CET) of plunge-frozen whole bacteria and vitreous sections (CETOVIS), allow visualization of biological samples close to their native state, enabling us to refine our knowledge of already known bacterial structures and to discover new ones.

**Objectives**

Application of these techniques to the Antarctic cold-adapted bacterium *Pseudomonas deceptionensis* M1<sup>T</sup> to demonstrate the existence of a previously undescribed cytoplasmic structure.

**Methods**

We used cryo-immobilization of specimens by rapid cooling followed by freeze substitution (FS) and sectioning, freeze fracture (FF) and observation of replica, cryoelectron microscopy of vitreous sections (CEMOVIS) and CET of plunge-frozen whole bacteria and vitreous sections to analyze this new structure.

**Conclusions**

*P. deceptionensis* M1<sup>T</sup> and other bacteria contain a new structure we termed 'Stack'. Each 'Stack' consisted of a pile of oval disc-like subunits, each disc being surrounded by a lipid bilayer membrane. They are found at different locations within the cell cytoplasm, in variable number, separately or grouped together, very close to the plasma membrane (PM) and oriented at different angles (from 35° to 90°) to the PM. Stacks did not appear to be invaginations of the PM. Regardless of their position, stacks were mostly observed very close to DNA fibers. This new structure deserves further study to refine its configuration and to establish whether its function could be related to chromosome dynamics.

**FEMS-2369**

**Microbial cell biology and structure**

**STRUCTURAL AND FUNCTIONAL INSIGHT IN THE *HELICOBACTER PYLORI* BLOOD GROUP ANTIGEN BINDING ADHESIN BAB A**

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**Background**

*Helicobacter pylori* is one of the most successful human pathogens, chronically infecting up to half the world population and thereby causing chronic gastritis, duodenal ulcers and even gastric cancer. The blood group antigen binding adhesin (BabA) of *H. pylori* mediates attachment to stomach glycoreceptors containing ABO and Lewis b blood group antigens.

**Objectives**

Our aim was to gain structural insight in the interaction between BabA and ABO/Lewis b blood group antigens.

**Methods**

BabA belongs to a paralogous family of *Helicobacter* outer membrane proteins (Hops) and we demonstrate BabA to feature a novel topology with an OmpA-like 8-stranded non-contiguous  $\beta$ -barrel that is disrupted by an extracellular domain that mediates the adhesive properties to BabA. The crystal and small angle X-ray scattering structure for the BabA extracellular domain reveals a two-domain architecture composed of an  $\alpha$ -helical head domain and a coiled-coil stem domain making the connection to the  $\beta$ -barrel domain. The X-ray structure of the BabA ectodomain bound to its natural glycan receptor Lewis b is presented. Receptor binding is located in a small subdomain grafted into the conserved head domain and critically depends on a disulphide-bound loop in the binding site. The ligand-bound structures of two BabA isomorphs provide a molecular rationalization of the observed blood group antigen polymorphisms in *H. pylori* clinical isolates.

**Conclusions**

Our results represent the first structural insight in how the important human pathogen *H. pylori* recognizes and interacts with its human host.

**FEMS-1009**

**Microbial cell biology and structure**

## **FUNCTIONAL STUDIES ON THE HMG-COA REDUCTASE GENES OF MUCOR CIRCINELLOIDES**

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### **Background**

Terpenoids have several biological functions. Among others, members of this family of chemical compounds are cell membrane components (e.g. ergosterol in fungi), pigments (e.g. carotenoids) or functional groups of certain proteins (e.g. RAS proteins). HMG-CoA reductase is a membrane anchored protein catalysing the central step of the acetate-mevalonate pathway of the terpenoid biosynthesis. Genome of the carotenoid producing zygomycete fungus *Mucor circinelloides* contains three HMG-CoA reductase genes (i.e. *hmgR1*, *hmgR2* and *hmgR3*). Earlier, we analysed the expression of these genes under different growth conditions by qPCR studies (1).

### **Objectives**

In this study, function of these genes and subcellular localization of the encoded proteins were analysed. Functional studies were carried out by overexpression and silencing the three genes.

### **Methods**

For silencing, the antisense RNA technique was used. Carotene and ergosterol content and morphology of the overexpressed and silenced transformants were examined. To analyse the subcellular localization of the enzymes, they were fused with the green fluorescent protein and different dyes were used to analyse their co-localization to different compartments of the cell.

### **Conclusions**

Our results suggest that HMGR2 and HMGR3 co-localize to the endoplasmic reticulum. HMGR2 plays role in the general isoprenoid biosynthesis, while HMGR3 is especially active under anaerobic growth condition and participates in the morphogenesis and the germination of the spores.

This study was supported by the grant OTKA NN106394 and the Hungarian-German Intergovernmental S & T Cooperation Programme TÉT\_12\_DE-1-2013-0009.

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**FEMS-0695**

**Microbial cell biology and structure**

## **OUTER-INNER MEMBRANE VESICLES NATURALLY SECRETED BY GRAM-NEGATIVE PATHOGENIC BACTERIA**

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### **Background**

Outer-inner membrane vesicles (O-IMVs) were recently described as a new type of membrane vesicle (MV) secreted by the Antarctic bacterium *Shewanella vesiculosa* M7<sup>T</sup>. Their formation is characterized by the protrusion of both outer and plasma membranes, which pulls cytoplasmic components into the vesicles [1].

### **Objectives**

The aim of the current study was to determine whether these new O-IMVs are also secreted by pathogenic Gram-negative bacteria whose MVs have been repeatedly reported to contain cytoplasmic components and DNA.

### **Methods**

For this purpose, three pathogenic bacteria: *Pseudomonas aeruginosa* PAO1, *Acinetobacter baumannii* AB41 and *Neisseria gonorrhoeae* DSM15130 and their derived MVs were analyzed by Transmission Electron Microscopy (TEM) and Cryo-TEM. The presence of the cytoplasmic constituents such as DNA and ATP was also evaluated. The encapsulation of DNA inside the O-IMVs was confirmed by gold DNA immunolabeling. A proteomic analysis of *N. gonorrhoeae*-derived membrane vesicles was performed to identify proteins from the cytoplasm and plasma membrane.

### **Conclusions**

TEM and Cryo-TEM techniques enabled us to demonstrate that Gram-negative pathogenic bacteria produce the recently described new type of vesicles, O-IMVs, together with conventional outer membrane vesicles (OMVs). The existence of O-IMV extends the hitherto uniform definition of MVs in Gram-negative bacteria and explains the presence of components in MVs such as DNA, cytoplasmic and inner membrane proteins, as well as ATP [2].

### **References**

1. **C. Pérez-Cruz et al.** 2013. AEM. **79**: 1874–81. DOI: 10.1128/AEM.03657-12.
2. **C. Pérez-Cruz et al.** 2014. Outer-Inner Membrane Vesicles Naturally Secreted by Gram-negative Pathogenic Bacteria. Plos one (Accepted).

**FEMS-2543**

**Microbial cell biology and structure**

## **ADAPTATION OF THE BACTERIAL CHEMOTAXIS SYSTEM TO THE ARCHAEAL MOTILITY MACHINERY**

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### **Background**

The ability to sense and respond to external stimuli is a key feature of all living organisms. Motility is essential to achieve this goal. Archaea are able to swim using a rotating surface structure, the archaellum. It functions analogous to the bacterial flagellum, but its components are unrelated. Instead the archaellum subunits are homologous to components of type IV pili. In bacteria, transfer of external stimuli to the flagellum is mediated by the chemotaxis system. The response regulator CheY plays herein a central role. Euryarchaea display photoaxis, aerotaxis and chemotaxis. Euryarchaea have acquired chemotaxis components from bacteria via horizontal gene transfer. This raises the question how the bacterial chemotaxis machinery has been evolutionary adapted in order to interact with subunits of the archaeal motility structure.

### **Objectives**

We aim to elucidate the functional and structural details interaction of the archaeal CheY with the archaellum motor complex to alter the direction of swimming.

### **Methods**

We have applied crystallography to reveal the structure of the euryarchaeal CheY in 1.6 Å resolution. In addition we have determined its phosphorylation state, as well as that of CheA. CheA is an upstream chemotaxis signaling component, which in bacteria is responsible for phosphorylation of CheY.

### **Conclusions**

The crystal structure of the archaeal CheY shows high similarity to the bacterial CheY. However, the interface of flagellum interaction is very basic in the bacterial CheY, while this same site shows a negative charge in the archaeal CheY. This suggests that the archaeal CheY is structurally adapted for interaction with the archaellum.





**FEMS-2461**

**Microbial cell biology and structure**

## **IDENTIFICATION OF NOVEL BINDING PARTNERS OF CAMPYLOBACTER JEJUNI FLAGELLINS**

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### **Background**

Flagella are propeller-like structures embedded in the bacterial envelope, which allow bacteria to swim in directions dictated by diverse sensory signals. In many pathogenic bacterial species, flagellar motility is required to reach the ultimate infection niche and to establish infection. The human pathogen *Campylobacter jejuni* is a highly motile organism that possess a flagellum on each pole. As many as 20,000 subunits of flagellin are assembled into a single filament *via* highly regulated process. Most flagellar proteins are bound to specific chaperones before export by the flagella assembly machinery. *C.jejuni* encodes for three different flagellins, however flagellar chaperones have not been characterized yet.

### **Objectives**

Flagellar chaperones are well-studied in Gammaproteobacteria but not Epsilonproteobacteria including the human pathogens: *Campylobacter jejuni* and *Helicobacter pylori*. The goal of this study is to characterize the flagellar chaperones in *C.jejuni*, the main cause of bacterial food-borne infections.

### **Methods**

Potential flagellar chaperones of *C.jejuni* were cloned and expressed in *E. coli*. Interaction of the recombinant putative chaperones with purified *C.jejuni* FlaA flagellin was tested via *in vitro* and *in vivo* pull-down experiments. The effect of targeted mutagenesis of the chaperones on flagella production was tested by a motility soft agar assay and (electron)microscopy methods. Western-blotting and qRT-PCR were used to detect differences in the FlaA, FlaB or FlaC flagellin expression levels.

### **Conclusions**

Targeted mutagenesis of putative flagellar chaperones influenced bacterial motility, flagellin levels and flagella length. The observed phenotype can be explained by direct interaction of chaperones with FlaA flagellin as demonstrated by pull-down experiments.

**FEMS-0935**

**Microbial cell biology and structure**

**LEA PROTEINS ARE INVOLVED ON DESICCATION RESISTANCE AND OTHER ABIOTIC STRESSES IN AZOTOBACTER VINELANDII**

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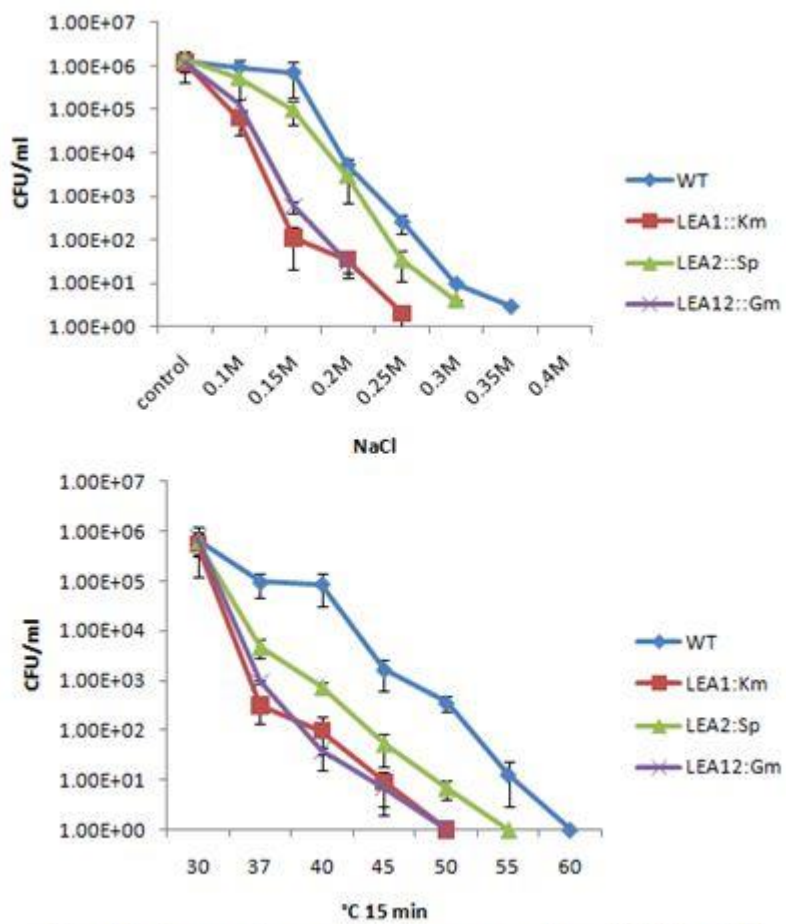
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**Background**

In plants, the expression of Late Embryogenesis Abundant Proteins (LEA) correlates closely with the acquisition of tolerance against drought, freezing and salinity stresses. In *A. vinelandii* we found two genes with homology sharing identity to putative LEA proteins from *Arabidopsis thaliana*, the sequence of the genes contains a part of the LEA eukaryotic motif, but differs from the distinctive motive found in other bacteria, so this could be an example for the possibility of horizontal gene transfer between domains of life.

**Objectives**

The aim of this work is to analyze if LEA proteins are involved on the resistance to desiccation, heat, freezing and osmotic stress in both cysts and vegetative cells of *A. vinelandii*.



**Fig. 1** Tolerance to osmotic stress caused by a) NaCl and b) Sorbitol in *A. vinelandii* cells

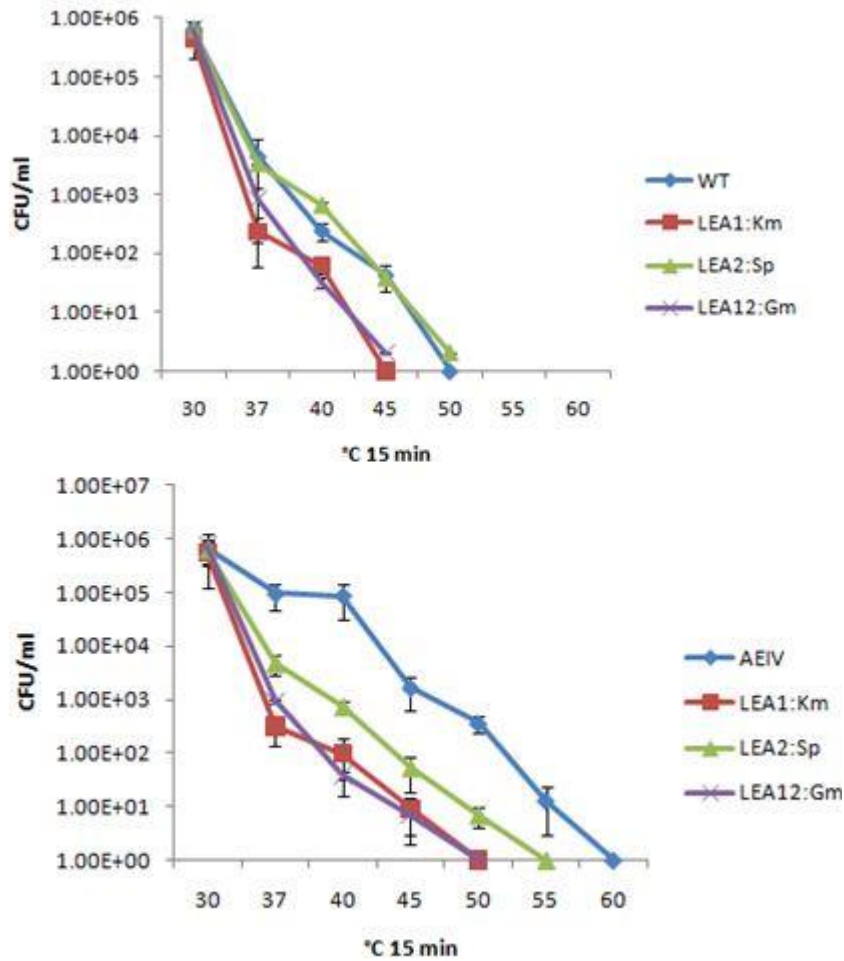


Fig. 4 Tolerance to high temperatures in a) vegetative cells , b) cysts of *A. vinelandii* .

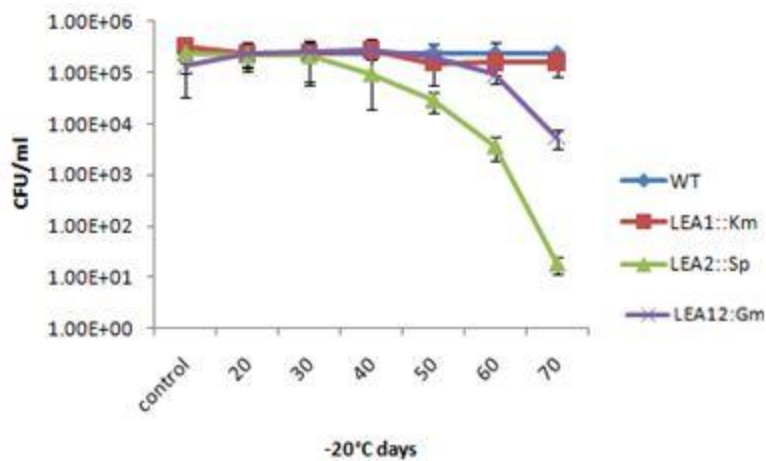


Fig. 5 Tolerance to freezing in *A. vinelandii* vegetative cells.

% Desiccation Resistant Cysts

Strain	WT	LEA1
1	9.52%	1.40%
2	16.23%	0.0%
3	7.25%	2.30%
Mean	11.00%	1.23%

## Methods

We constructed mutants for these genes, LEA1 and LEA2 and a double mutant LEA12. Cysts and vegetative cells were treated to desiccation for 3 months; osmotic stress was caused by different concentrations of NaCl and Sorbitol. We tested the tolerance of these mutants to high temperatures (60°C for 15 minutes) and freezing (-20°C for three month) for both cysts and vegetative cells.

## Conclusions

LEA proteins are necessary to confer tolerance to desiccation on cyst cells after 3 months treatment and are necessary to survive at high osmotic agents concentrations in vegetative cells too. LEA proteins can also protect cyst and vegetative cells against high and low temperatures since LEA mutants were unable to grow at 50°C or -20°C

**FEMS-2459**

**Microbial cell biology and structure**

**STRUCTURE AND MECHANISM OF THE BACTERIAL AMYLOID SECRETION CHANNEL CSGG**

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**Background**

Curli are proteinacious fibers that constitute the major component of the extracellular matrix in pellicle biofilms in many Gram-negative bacteria.

**Objectives**

Numerous fundamental questions about the curli biogenesis pathway remain unsolved. Curli formation requires a dedicated protein secretion machinery comprised of the outer membrane lipoprotein CsgG responsible for the transport of curli subunits across the outer membrane and two soluble accessory proteins.

Here we propose a novel diffusion-based and entropy-driven transport mechanism that is as a working model for the CsgG translocator.

**Methods**

Recently the X-ray structure of this protein was solved within our group, revealing a nonameric complex embedded in the outer membrane by a 36-stranded  $\beta$ -barrel. Based on this structure and pre-eliminary single-channel results we observe that the CsgG pore on its own is an ungated, non-selective protein secretion channel. As earlier described in literature we confirm that the addition of certain amounts of the CsgE chaperon protein blocks the CsgG pore.

**Conclusions**

Together these findings support the working model we propose. Future structural and single-channel analysis give even more insight in the process and will probe how subunit secretion is coupled with fiber assembly.

**FEMS-1319**

**Microbial cell biology and structure**

**GACA IS ESSENTIAL FOR GROUP A STREPTOCOCCUS AND DEFINES A NEW CLASS OF MONOMERIC dTDP-4-DEHYDRORHAMNOSE REDUCTASES**

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**Background**

Group A *Streptococcus* (GAS) is a preeminent Gram-positive human pathogen. GAS expresses a characteristic cell wall polysaccharide, known as Group A Carbohydrate (GAC), consisting of rhamnose and *N*-acetylglucosamine (GlcNAc), which is critical for full virulence. The final step of the four-step dTDP-L-rhamnose biosynthesis process is catalyzed by dTDP-4-dehydrorhamnose reductase (RmlD) in an NAD(P)H-dependent manner. Previous structure elucidation of RmlD from *Salmonella enterica* demonstrated that Mg<sup>2+</sup>-dependent dimerization is essential for enzymatic activity. The first gene in the GAC gene cluster, *gacA*, is annotated as an RmlD enzyme.

**Objectives**

We aimed to identify the structure, function and biological importance of the *gacA* gene product.

**Methods**

We generated recombinant GacA protein for biochemical and structural analysis. Also, a saturated transposon library of GAS was screened by whole genome sequencing (Tn-seq) and subjected to Bayesian analysis to identify essential genes. Finally, *gacA* was expressed in a *S. mutans rmlD* knockout to analyse whether this would restore its attenuated phenotype.

**Conclusions**

GacA catalyses the biosynthesis of dTDP-rhamnose in a novel Mg<sup>2+</sup>-independent monomeric manner. The 1.1 Å high-resolution structure of GacA confirms conservation of key active site residues, but GacA lacks the dimerization motif characteristic for RmlD in Gram-negative bacteria. GacA may therefore represent a new class of monomeric RmlD enzymes. We identified *gacA* as an essential gene and confirmed enzymatic function by restoring the growth and morphology of an *S. mutans rmlD* mutant. Our report provides a framework for future screenings to identify novel inhibitors that interfere with dTDP-rhamnose biosynthesis in human pathogens.

**FEMS-2167**

**Microbial cell biology and structure**

**CYDIV, A CRITICAL PROTEIN IN THE CELL DIVISION IN ANABAENA SP. PCC7120.**

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**Background**

Bacterial cell division is a fundamental and complex process that requires coordinated mechanisms for elongation and septal formation in the middle of the cell. Most researches in cyanobacterial division have been focused on identifying proteins that interact specifically with the FtsZ protein, also revealing that the division machinery presents differences in composition and regulation compared to the division of gram-negative and gram-positive model microorganism. Studies carried out in our laboratory have determined a gene common to all filamentous cyanobacteria (cyDiv), which encodes a protein with one transmembrane and coiled-coil domains. The phenotype of the mutant strain in this gene presents serious defects in cell division.

**Objectives**

To determine the participation of CyDiv protein in the cell division process of the filamentous cyanobacteria *Anabaena* sp. PCC7120.

**Methods**

We analyzed CyDiv localization by immunofluorescence generating specific polyclonal antibodies against the protein from a synthetic peptide. Furthermore, using bioinformatic analyses, we predicted the possible role of this protein.

**Conclusions**

We showed that CyDiv is localized in the cell poles and in the middle of the cell depending on the division phase, co-localizing with the Z-ring. These results, together with previous phenotypical observations of the cyDiv mutant, suggest that CyDiv is essential for cell division in filamentous cyanobacteria. In silico analyses determined that this protein has a similar topology to the cellular division proteins FtsB and DivIC, from *E. coli* and *B. subtilis* respectively.

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**FEMS-0954**

**Microbial cell biology and structure**

**BIOPHYSICAL CHARACTERIZATION OF A TRIMERIC AUTOTRANSPORTER  
ADHESIN, ATAA, FROM THE HIGHLY ADHESIVE BACTERIUM  
ACINETOBACTER SP. TOL 5**

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**Background**

The Gram-negative bacterium *Acinetobacter* sp. Tol 5 shows autoagglutinating nature and noteworthy adhesiveness independent of biofilm formation through a trimeric autotransporter adhesin (TAA), namely AtaA. TAA is one of the major outer membrane proteins widely distributing in Gram-negative bacterial species. Many studies have shown that TAAs from pathogens adhere to ECM proteins, such as collagen, fibronectin and laminin. In contrast, we previously reported that AtaA mediates bacterial cell adhesion to various abiotic surfaces nonspecifically. However, we have investigated the adhesive property mediated by AtaA only in a cellular level.

**Objectives**

Here, we report the characteristics of AtaA as a molecule. We attempted to purify the AtaA molecule and characterize its biophysical and adhesive properties.

**Methods**

The structural stabilities in thermal treatment and pH change were analyzed by transmission electron microscopy (TEM) and circular dichroism (CD). The adhesion property of the purified AtaA molecule was analyzed by quartz crystal microbalance (QCM).

**Conclusions**

The TEM images of the purified AtaA showed slightly curved fiber structure of 225 nm in length with two globular domains at the amino-terminal and near the carboxyl terminal. The adhesive function of the purified AtaA remained after pH treatment. In contrast, that of the purified AtaA decreased with disruption of its structure after thermal treatment more than 80°C. These results suggest that AtaA molecule has relatively high resistance against strict conditions and its fiber structure plays crucial role in its adhesiveness to abiotic surfaces.

**FEMS-2316**

**Microbial communities**

**ISOLATION AND IDENTIFICATION OF NEW HALOPHYTE AND HALOTOLERANT STRAINS FROM THE RHIZOSPHERE OF ARTHROCNEMUM MACROSTACHYUM PLANTS COLLECTED FROM LEBRIJA MARSHES (SW- SPAIN)**

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**Background**

Halophytes are highly salt tolerant plants able to survive and flourish in coastal salt marshes and salt steppes. Lebrija marshes are situated at SW-Spain (36° 64' 29" N - 6° 12' 92" O), covering an area of 13000 has. Several halophytes are able to grow in this zone. Among them, we have selected *Arthrocnemum macrostachyum*, a plant with proven desalination capacity, as source for isolating halotolerant rhizosphere microorganisms.

**Objectives**

Isolate halotolerant rhizosphere microorganisms.

**Methods**

A total of two hundred bacteria have been isolated from plants grown in two different zones of the Lebrija-marshes at two different seasons (autumn and summer). These strains have been tested for their capacity to grow under high salinity conditions and six strains have been selected.

Rhizospheric soil samples were suspended and homogenised in sterile mineral buffer. Serial dilutions were plated on Nutrient Agar supplemented with 300 mM NaCl and cycloheximide (100 mg/l), adjusted at pH 7.4, at 28 °C for 5 days.

**Conclusions**

Three of the six strains, named Cs16b, Cs25 and Hvs 18 were unable to grow without salt in the culture media (halophyte strains) being the last one (Hvs18) able to support up to 20% NaCl. The other strains (Hv14b, Hv16 and C14) were halotolerant ones. All of them have been sequenced and characterized. Cs16b, Cs25 and Hv16 have been described as novel species within the genera *Microbulbifer*, *Labrenzia* and *Kokuria*, respectively.

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**FEMS-1743**

**Microbial communities**

## **EFFECT OF WOOD CREOSOTE ON INTESTINAL MICROBIOTA IN RATS**

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### **Background**

Diarrhea is influenced on specific pathogenic microbes. Wood creosote is an anti-diarrhea medicine to have been used for about 100 years in the northeastern Asia.

### **Objectives**

We investigated the change of intestinal microbiota in rats to be fed with wood creosote.

### **Methods**

Sixty rats (7 weeks old) were divided to 5 groups; control, antibiotic group, 0.2%, 0.4% and 0.8% wood creosote groups. The wood creosote and the antibiotic were supplied with feed for 4 weeks. To analyze intestinal microorganisms, the pyrosequencing was performed with 16S rRNA genes of microorganisms from intestinal contents.

The control group distributed 6 phyla including 229 species of microorganisms, the antibiotic group 7 phyla including 245 species, 0.2% wood creosote group 7 phyla including 284 species, 0.4% wood creosote group 7 phyla including 276 species and 0.8% wood creosote group 7 phyla including 324 species, respectively. *Clostridium* to belong to *Clostridia* that include many species of pathogenic microorganisms was 33.4, 44.3, 37.0, 35.5 and 11.6 per cents in the control group, the antibiotic group, 0.2, 0.4 and 0.8% wood creosote groups, respectively. The wood creosote dose-dependently decreased *Clostridium* in the large intestine.

### **Conclusions**

We think that the supply of the wood creosote increases the diversity of the intestinal microorganisms and inhibits the propagation of specific pathogenic microorganisms.

**FEMS-2378**

**Microbial communities**

## **DEEPWATER HORIZON SPILL'S IMPACT ON SHIPWRECK MICROBIOMES AND SURROUNDING ENVIRONMENT**

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### **Background**

Shipwrecks serve as artificial reefs in the deep ocean. Due to their random distribution and inherent diversity compared to the surrounding environment, shipwrecks are ideal ecosystems to study pollution impacts, microbial distributions and marine corrosion.

### **Objectives**

To comparatively assess the *Deepwater Horizon* spill's impacts on shipwreck microbiomes and the synergistic effects on these communities and surrounding environment. Field activities will generate material and environmental samples required to address questions related to contaminant effects resulting from the spill and the interaction of spill related contaminants on the microbiota. Microbial action at each site will be interrogated to determine if exposure to hydrocarbons and dispersant-based chemicals have impacted the artificial reefs.

### **Methods**

Porewater from sediment samples were analyzed by ion chromatography and inductively coupled plasma optical emission spectrometry to quantify major elements. Concentration and stable isotope ratios for sediments were determined using an elemental analyzer interfaced to an Isotope-Ratio Mass Spectrometer (IRMS). The molecular analysis of sediment from wreck sites was performed on the Ion Torrent Personal Genome (PGM) machine to provide a comprehensive assessment of microbiome composition. An analytical pipeline to extract taxonomic information from PGM sequences was completed to provide metabolic functional information from DNA sequences.

### **Conclusions**

Six shipwrecks in the northern Gulf of Mexico were investigated. Various laboratory tasks have been completed since the second field research expedition funded by this project. Taxonomic classification of shipwreck microbiomes and metabolic information extracted from sequence data yielded new understanding of microbial processes associated with shipwrecks in the deep biosphere.



**FEMS-0831**

**Microbial communities**

**PREDICTING THE DYNAMICS AND BIODEGRADATION POTENTIAL OF RIVER MICROBIAL COMMUNITIES IN ANTHROPIZED WATERSHEDS: A WET LAB APPROACH.**

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**Background**

River waters represent less than 0.0002 % of global water resources but they are essential for human life. Yet they are particularly exposed to anthropogenic stresses and risks of degradation. In a context of diminishing access to clean resources, lotic microbial communities play a key role in water self-purification. However they remain largely uncharacterized, and the link between lotic bacterial diversity and bacterial community stability and performance has not been studied.

**Objectives**

The goal of our project is to determine which factors drive the response of lotic microbial communities to organic pollution and how they tolerate, resist, exploit this pollution and/or go back to original status (resilience) downstream sewage outfalls. More specifically, the role of (i) native community structure (both phylogenetic and functional), (ii) wastewater bacteria released with organic matter and (iii) type of disturbance (regular/occasional, low/high loads) are studied using bacterial strains isolated from the Zenne river, Belgium.

**Methods**

Random artificial assemblages of these strains are used to test the effect of microbial community structure (richness, evenness, architecture, metabolic diversity...) on community performance and recovery from stress.

**Conclusions**

We hypothesize that such simplified, “wet lab” approaches can help to develop predictive tools of the dynamics and activity of bacterial communities in urban watersheds and *in fine* contribute to a better protection and restoration of river ecosystem services.

**FEMS-2939**

**Microbial communities**

**METATAXONOMIC PROFILING AND METABOLIC POTENTIAL OF TWO SOIL-DERIVED MICROBIAL CONSORTIA SELECTED ON WHEAT STRAW**

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**Background**

Mixed microbial cultures have been proposed as an efficient way to deconstruct plant waste. The characterization of specific microbial consortia could be the starting point for novel biotechnological applications related to the efficient conversion of lignocellulose to cello-oligosaccharides, plastics and/or biofuels

**Objectives**

Here, the diversity, composition and functional profiles of two novel microbial consortia are reported, on the basis of replicated aerobic wheat straw enrichment cultures

**Methods**

Soil as microbial source and three sequential-batches were evaluated by amplicon-pyrosequencing (bacterial 16S rRNA and fungal ITS1) and whole metagenome sequencing

**Conclusions**

Both systems appeared to indeed incite a reshaping of the bacterial communities, with reductions in richness and increases in prevalence of particular members of the Enterobacteriales, Pseudomonadales, Flavobacteriales and Sphingobacteriales. Among the fungal players with high biotechnological relevance, co-existing with bacteria ones, we detected member of the genera *Coniochaeta*, *Acremonium* and *Trichosporon*. Metagenomic analyses revealed an overrepresentation of diverse carbohydrate transporters, plant polysaccharides sensing proteins and several genes encoding enzymes of the glycosyl hydrolase families GH2, GH43, GH92 and GH95 in the two consortia, as compared to the soil inoculum. These analyses are the starting point for synthetic expression systems and could enhance our understanding of microbial lignocellulose bioconversion in engineered or natural soil environments



**FEMS-0801**

**Microbial communities**

### **MYCORRHIZAS AND THE ECOLOGY OF FOREST SUCCESSION**

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### **Background**

Identifying the drivers of plant community succession is a central theme in ecology. Research has shown that plant-soil feedbacks drive community assembly and has inspired many theories of species diversity. An important unsettled issue is whether mycorrhizas mediate plant-soil feedbacks to determine tree community succession.

### **Objectives**

We show that ectomycorrhizas (but not arbuscular mycorrhizas) define interactions among trees and therefore have the ability to trigger and eventually to arrest tree community succession.

### **Methods**

Using reciprocal invasion design with ectomycorrhizal mycorrhizal (EcM) and arbuscular mycorrhizal (AM) tree species, we tested for the potential for shared compatible mycorrhizal fungi to modulate interactions between invading seedlings ('daughters') understorey and resident trees (mycorrhizal 'mothers') in replicate mesocosms.

### **Conclusions**

Characterizing mycorrhizal fungal communities using pyrosequencing, we find that EcM seedlings favored EcM fungi over AM fungi, but AM seedlings exhibited no mycorrhizal preference. The results from growth survey and physiochemical data confirm (i) invasibility of EcM fungi (and hence EcM seedlings) to AM-dominated communities, (ii) detrimental effects of EcM fungal diversity on AM daughters and mothers, and (iii) the ability of EcM fungal network in reducing the relative growth of EcM daughters to EcM mothers. The results demonstrate that invasion of EcM fungi facilitates establishment of EcM trees on the presence of AM trees, and eventually arrests the relative growth of EcM daughters to EcM mothers, a relationship potentially regulating tree community succession. The observed shifts from positive to negative plant-soil feedbacks involving EcM may explain why the climax temperate forests are predominantly ectomycorrhizal.



**FEMS-1559**

**Microbial communities**

## **MICROBIAL INTERDEPENDENCIES IN AN ENRICHED AEROBIC MIXED METHANOTROPHIC COMMUNITY**

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### **Background**

Aerobic methanotrophic bacteria are capable of both energy conservation and growth with methane as carbon source. Next to this unique feature they can be primary producers, supporting microbial foodwebs, particularly in aquatic environments. Recently it has been suggested that disparate microbial populations, including the methanotrophs are responsible for methane cycling rather than individual methanotrophs. The interactions between methanotrophs and heterotrophic partners have also been shown to be highly specific and beneficial to the rate of methane oxidation. Methane is an important greenhouse gas and methanotrophic communities could be used for both mitigation and biotechnological applications, warranting investigations into their composition.

### **Objectives**

The aim of this work was to gain insight in the primary and secondary consumers from an active laboratory methanotrophic enrichment culture.

### **Methods**

A methanotrophic mixed culture from various sources was incubated with both <sup>13</sup>C labeled methane and <sup>12</sup>C methane. At several points in time the 16S rRNA genes of the community were amplicon sequenced from both the light and heavy fractions to identify the first partners to incorporate labeled methane (methanotrophs and primary consumers) and later partners (secondary consumers). Interesting timepoints were selected based upon denaturing gradient gel electrophoresis profiles at 24 and 60 hours of incubation.

### **Conclusions**

The main methanotrophic community driver was *Methylophilus* sp. Unique primary partners in the heavy fraction at 24h were members of the genera *Acidivorax*, *Curvibacter*, *Pseudoxanthomonas*, *Methylophilus* and *Rhodobacter*. At 60h the genera *Sphingobacter*, *Kaistia*, *Sphingomonas*, *Flavobacterium*, and *Methylobacillus* represented the heavy fraction. Isolation of these partners from the community could help elucidate their influence on methane cycling.

**FEMS-1312**

**Microbial communities**

## **IMPROVEMENT OF SEQUENCE-BASED BACTERIA IDENTIFICATION IN POLYBACTERIAL SPECIMENS BY RIPSEQ MIXED SOFTWARE**

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### **Background**

Sequencing-based molecular methods have become a substantial tool in microbiology but analysis of polybacterial samples still remains a methodological challenge. RipSeq Mixed represents software-based technique, when up to 3 mixed chromatograms can be separated *in silico*, however this possibility can be inadequate for some types of microbiological material.

### **Objectives**

We present the use of Denaturing Gradient Gel Electrophoresis (DGGE) followed by sequencing in combination with analysis of polymicrobial sequences by RipSeq Mixed software. Combination of these methods was used for detection of bacteria in urine and ureteral stent sonicates. Bacteria were detected also by culture-dependent techniques.

### **Methods**

A total of 45 specimens of 15 patients were collected. The V3-V5 region of the 16S *rRNA* gene was amplified and multiple length homogeneous amplicons were separated by DGGE. Bands were excised and reamplified PCR products were sequenced. RipSeq Mixed software was used in case of mixed chromatograms.

### **Conclusions**

There were 40 PCR-positive specimens (80 % of all specimens), 32 specimens were able to analyze only with PCR-DGGE. Ten bands (20 % of all bands) exhibited mixed nature, so that 8 specimens were identified by the combination of PCR-DGGE and RipSeq Mixed. The combination of established PCR-DGGE method and RipSeq Mixed analysis can eliminate failures of both approaches. More than 3 bacteria in a specimen can be detected and mixed chromatograms can be separated.

**FEMS-1085**

**Microbial communities**

## **SOIL METHANOTROPHIC COMMUNITIES IN AN ALPINE WETLAND OF THE TIBETAN PLATEAU**

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### **Background**

Methane (CH<sub>4</sub>) plays an important role in global warming and has been responsible for approximately 20% of the Earth's warming since the pre-industrial period. However, most CH<sub>4</sub> is consumed by methanotrophs before its emission into the atmosphere.

### **Objectives**

To understand the methane oxidizers in wetlands, the largest source habitat of atmospheric CH<sub>4</sub>.

### **Methods**

We used quantitative qPCR and functional gene-targeted 454 pyrosequencing to elucidate the abundance and diversity of aerobic and anaerobic (N-DAMO) methane oxidizers in the Zoige Wetland, which is located on the Tibetan Plateau and is the largest alpine wetland in the world.

### **Conclusions**

Our results demonstrated that type I methanotrophs were the primary aerobic methanotrophs, and *Methylobacter* comprised approximately 50% of the community. Type II methanotrophs, represented by the genus *Methylocystis*, were also detected, and the presence of N-DAMO bacteria in the Zoige Wetland was confirmed by the quantification and pyrosequencing analyses of N-DAMO 16S rRNA and *pmoA* genes. Furthermore, aerobic *pmoA* gene ranged from 10<sup>7</sup> to 10<sup>8</sup> copies•g<sup>-1</sup> of fresh soil, and the 16S rRNA gene of the NC10 phylum ranged between 10<sup>4</sup> and 10<sup>5</sup> copies•g<sup>-1</sup> of fresh soil. The results suggest that the aerobic and anaerobic methane-oxidizing communities together affect CH<sub>4</sub> cycle at the Zoige Wetland.

**FEMS-2739**

**Microbial communities**

## **TRACKING RELATIONS AMONG BACTERIAL AND PROTOZOAN COMMUNITIES IN WASTEWATER TREATMENT PLANTS**

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### **Background**

Understanding the interactions between prokaryotic and eukaryotic populations in complex environments can be challenging. Particularly, there is a recognized difficulty in establishing how the interactions between the bacterial and the protozoan populations can affect the performance of wastewater treatment plants (WWTP).

### **Objectives**

In order to determine the relationships between microbial communities (protozoa, metazoa and filamentous bacteria) and abiotic parameters (physical-chemical and operational), thirty seven WWTP with activated sludge process were studied in Portugal, during two years, in a trimestral sampling strategy.

### **Methods**

Samples were collected to enable a molecular characterization of the microbiota. In order to ensure the diversity of microorganisms, 100 samples were selected based on their geographic localization and time-spaced to track relationships within bacteria and protozoa. A 16S rRNA gene PCR-DGGE approach was carried out for bacterial community fingerprinting. The resulting profiles were analysed together with the results obtained from the survey of the protozoa populations. Finally, the study of the correlations between the physical-chemical and operational parameters and the microbial communities was also investigated.

### **Conclusions**

The study of these interactions constitutes a strategy for the understanding of how the different metabolic groups of prokaryotic and protozoa are affected by the relations between this populations, allowing a better prediction of the overall community dynamics. This will allow setting the conditions that better contribute to improve the wastewater treatment while increasing the knowledge of overall communities networking in complex environments.

## **FEMS-1104**

### **Microbial communities**

#### **INTRA-AMNIOTIC AND VAGINAL MICROBIOMES AT THE END OF PREGNANCY**

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#### **Background**

The microbiota of the genital tract of *pregnant women* remains to be elucidated. Recent studies of the bacterial populations in different groups of pregnant women have yielded considerable amount of information on their bacterial communities. However, few comparisons have been performed between the vaginal and intra-amniotic microbiomes of individual women, and of women of different racial backgrounds.

#### **Objectives**

To investigate the compositions of the vaginal and intra-amniotic bacterial populations in late pregnancy of women from different racial backgrounds, and to compare the compositions of their genital microbiomes.

#### **Methods**

Vaginal and intra-amniotic swabs were obtained from 90 women at delivery by Caesarean section. Participants were administered a questionnaire including demographic and pregnancy data. The identity of the taxa present was determined by ultrafast sequencing of the V3V5 regions of the 16S rDNA gene of samples. Species diversity and richness, and Shannon's diversity analyses were performed on the sequence data of each woman. SIMPER analysis showed the contribution of each species to the vaginal and intra-amniotic microbiomes. PERMANOVA and PERDISP analyses showed that both genital microbiomes depend on racial background.

#### **Conclusions**

At variance with commonly held assumptions, the analyses identified in the intra-amniotic cavity of the women in the study rich and diverse bacterial populations that were significantly different to the vaginal populations. The results suggested that the intra-amniotic microbial profile remains quite stable independently of the variability of the vaginal microbial profile. The outcomes of this study have potentially important implications for the management of urogenital infections during pregnancy.

**FEMS-2528**

**Microbial communities**

**METABOLIC TRANSFORMATIONS ENHANCES PSEUDOMONAS AERUGINOSA GROWTH ACTIVITY DURING INTERACTION WITH STAPHYLOCOCCUS AUREUS**

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**Background**

Interactions among members of polymicrobial infections can result in altered pathogen behaviours such as enhanced virulence, biofilm formation or antibiotic tolerance, which may influence the disease phenotype and clinical outcome of the infection. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are important opportunistic human pathogens and are both part of the polymicrobial infection communities in human hosts. Cell-cell communication can play a major role in the interaction behaviors between these bacteria, which subsequently can affect the physiology of the individual strains. However, the extent to which evolutionary processes may remodel interspecies interactions and bacterial physiology during the course of infection and therapy is currently not understood.

**Objectives**

Therefore, the aim of this study was to analyze *in vitro* interactions between *S. aureus* and a human host adapted *P. aeruginosa* strain from a dominant lineage, DK2, that have evolved through decades of growth in chronically infected patients.

**Methods**

By using a combination of *in vitro* agar assays, molecular genetics and MALDI-TOF imaging mass spectrometry we demonstrate that DK2 – in contrast to other *P. aeruginosa* strains - show a commensal-like interaction with *S. aureus* during *in vitro* co-culturing, where the growth activity of *P. aeruginosa* was increased in the presence of *S. aureus*. The commensal interaction was observed both under aerobic and anaerobic conditions as well as during growth on artificial CF sputum medium, however was abolished with *S. aureus* strains mutated in the *agr* quorum sensing system.

**Conclusions**

Our results suggest that metabolic transformations during interaction with *S. aureus* enhance the growth activity of *P. aeruginosa*.





**FEMS-2984**

**Microbial communities**

## **OCCURRENCE AND ACTIVITY OF METHANOGENIC ARCHAEA IN AGRICULTURAL BIOGAS PLANTS**

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### **Background**

Anaerobic digestion of organic substrates is a complex process which is accomplished by the cooperation of different microorganisms. The last part of the biogas process, the methanogenesis, is mostly the bottle neck of the whole process and is still not completely understood. Recently, molecular analyses of the microbial populations shed light on the microbiological 'black box', but on DNA-level, the whole population consisting of dead and inactive cells is analyzed

### **Objectives**

In this study, biogas plants were analyzed to investigate which fractions of the methanogenic community are transcriptionally active under different process conditions.

### **Methods**

Different lab- and full-scale biogas reactors with different substrate mixes and process conditions were analyzed for the community composition of methanogens on DNA- and mRNA-level. After DNA- and RNA-extraction, (RT-)PCR-cloning targeting the *mcrA/mrtA* gene coding for methyl-coenzyme M-reductase was conducted. Sequences were checked for chimeras, aligned and a phylogenetic tree was constructed.

### **Conclusions**

Hydrogenotrophic methanogens dominated in the investigated agricultural biogas plants, and acetoclasts were only present at high retention times. In well running systems only small differences were seen between the whole (DNA) and active (cDNA) methanogenic communities, and certain *Methanosarcinaceae* and *Methanobacteriaceae* dominated. During process disturbances, the active community mostly differed considerably from the whole community, and certain

*Methanobacteriaceae* took over the main transcriptional activity, not necessarily with productivity loss. A small part of the present methanogens obviously can take over the main part of the activity. The community should thus be analyzed on cDNA level in order to analyze the active part of the community reliably.

**FEMS-2072**

**Microbial communities**

## **PUBLIC HEALTH SURVEILLANCE OF NOSOCOMIAL INFECTIONS IN SOUTH-EAST, NIGERIA**

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### **Background**

Hospital-acquired infections (HAIs) are constituting major threat to hospitalization world-over. They occur in several different patient groups, especially patients on ventilators in intensive care unit where the rate of the pneumonia may be as high as 3% per day

### **Objectives**

This study was undertaken to unveil the burden of HAI in South-East, Nigeria.

### **Methods**

Structured questionnaires totaling 660 were administered personally to healthcare workers consisting of 96

doctors, 170 nurses, 24 pharmacists and 40 medical laboratory scientists from randomly selected government

hospitals and 90 doctors, 180 nurses, 30 pharmacists and 30 medical laboratory scientists from randomly selected private hospitals. Study was carried out between April and July 2013

The study shows that the prevalence of nosocomial infection in the hospitals surveyed is in the following

descending order: urinary tract infection (34.9%), *Staphylococcus aureus* (31.0%), Gastroenteritis (27.1%), Hospital acquired pneumonia (20.3%), *Candida albicans/Aspergillus spp* (10.8%), *Pseudomonas aeruginosa* (10.5%), Tuberculosis (9.0%), *Clostridium difficile* (3.9%), ventilator associated pneumonia (3.6%), Methicillin and vancomycin resistant bacteria (1.5%). The study also showed that the frequency of occurrence of HAP is higher in government than private hospitals. This can be attributed to the higher population of patients leading to overcrowding. The modes of transmission of hospital acquired pneumonia was observed to follow this order airborne > contact with blood and body fluids > contaminated instruments > contaminated hands > needle sticks.

### **Conclusions**

The prevalence of HAI in South-east, Nigeria is high. Efforts are needed to reduce the incidence, improve the quality of life of patients and healthcare workers and

improve healthcare delivery.

**FEMS-1124**

**Microbial communities**

## **TRACKING MICROBIAL COMMUNITY DYNAMICS IN ENGINEERED AQUATIC SYSTEMS THROUGH FLOW CYTOMETRIC FINGERPRINTING**

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### **Background**

Characterizing and monitoring **the dynamics of microbial communities** has become an important research field in microbial ecology. Yet conventional microbial (e.g. heterotrophic plate counts) and molecular (e.g. Denaturing Gradient Gel Electrophoresis) workflows are time consuming and laborious.

### **Objectives**

Here, we applied **a novel statistical pipeline** on **flow cytometry** data in order to perform **rapid high-resolution and objective monitoring** of the microbial community dynamics in an oligotrophic **engineered aquatic system**. The obtained results were then related to the imposed operational conditions (pH, temperature, conductivity).

### **Methods**

A full-scale engineered, oligotrophic **cooling water system** operating on a nuclear test reactor was used as model system. This system operated under 3 week cycles and was monitored throughout two full cycles by **flow cytometry** and **on-line physicochemical sensors**. Aside from generating phenotypic community fingerprints, flow cytometric analysis also allowed the assessment of **total cell counts, nucleic acid content and cell viability**. Additionally, the exact microbial community throughout the cycles was determined with 16S rRNA amplicon sequencing

### **Conclusions**

Community fingerprinting indicated **a highly dynamic community shift** during the operation of the cooling water system. Five distinct phenotypic communities related to start-up, operational periods and inactivity of the reactor were identified. Conductivity and temperature were the predominant physicochemical factors but only partially explained the observed community shifts. The cooling water microbial community further showed an **oscillating pattern in cell concentrations and nucleic acid content** on both weekly and daily scale. The output of this phenotypic “fingerprint” approach was validated using **high-throughput 16S rRNA amplicon sequencing**.



**FEMS-1444**

**Microbial communities**

## **COMPARATIVE ANALYSIS OF BACTERIAL DIVERSITY IN HEAVY METAL POLLUTED SOILS ESTIMATED BY MOLECULAR AND CULTIVATION-BASED TECHNIQUES**

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### **Background**

Pb-Zn smelter - KCM is the largest enterprise in the Balkan Peninsula producing lead, zinc and precious metals.

### **Objectives**

To assess bacterial diversity in heavy metal polluted soils *via* molecular and cultivation-based techniques.

### **Methods**

16S rDNA approach, as well as cultivation-based techniques were applied.

### **Conclusions**

Molecular analysis showed that microbial community in the studied samples consisted in different extent of *Proteobacteria* (a, b, g and d), *Actinobacteria*, *Holophaga/Acidobacteria*, AD1, *Cytophaga/Flavobacterium/Bacteroides*, *Cytophaga*, BD and novel bacteria. In the highly polluted soil sample - KCM 3 g-proteobacteria were not detected. Cultivation-based analysis showed presence of heterotrophic aerobs, heterophic anaerobs, sporeforming bacteria, denitrifying bacteria, amonifying bacteria, nitrifying bacteria, Fe(II)- oxidizing bacteria, Mn(II) oxidizing bacteria, Fe(III) redicing bacteria, Mn(IV) reducing bacteria, colourless sulphur bacteria, cellulose degradating bacteria, oligocarbophiles, metal-leaching bacteria *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus denitrificans*,



*Acidithiobacillus thioautotrophicus* except fungi and actinomycetes. Both analyses revealed that the microbial communities in the polluted area were strongly affected.

**FEMS-1446**

**Microbial communities**

**INFLUENCE OF VERY EARLY EXPOSURE TO CEFUROXIME VIA THE MOTHER ON GUT MICROBIOTA COMPOSITION IN TERM BABIES BORN BY CAESAREAN SECTION**

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**Background**

Cefuroxime is an antibiotic effective against a wide range of Gram negative and Gram positive bacteria. It is standard procedure to give the mother a single prophylactic dose (1500 mg) of cefuroxime in relation to caesarean section (CS). If the antibiotic is given before the umbilical cord is cut the antibiotic will pass from the mother to the infant. However, the effect of this very early exposure to cefuroxime on infant gut microbiota (GM) colonization is still not known.

**Objectives**

To investigate if very early exposure to cefuroxime in CS born infants influences GM colonization.

**Methods**

42 pregnant women (BMI<30) scheduled for elective CS were recruited at Odense University Hospital, Denmark. Two mothers were expecting twins meaning that 44 infants were included in the study. The mothers were randomly assigned to either receiving cefuroxime before skin incision or immediately after the umbilical cord were cut. Faecal samples were collected from all infants 10 days after birth and GM composition determined by MiSeq-based tag-encoded 16S rRNA gene targeted high throughput amplicon sequencing.

**Conclusions**

The GM of both groups were dominated by the genera *Bifidobacterium*, *Clostridium* (relative abundance of both genera ~5-10%), *Veillonella* (~25-30%) and family Enterobacteriaceae (~25-30%). Testing with analysis of similarity (ANOSIM) on both unweighted ( $p=0.872$ ,  $R=-0.0401$ ) and weighted ( $p=0.140$ ,  $R=0.0317$ ) uniFrac distance matrices analysis revealed no major differences between the 2 groups. In conclusion, early and short exposure to cefuroxime does not have major effects on infant GM colonization.

## FEMS-2092

### Microbial communities

#### QUANTITATIVE CHANGES OF CLOSTRIDIUM SP. IN HUMAN INTESTINAL TRACT OF DIFFERENT AGE GROUPS

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#### Background

Characterization of changes in gut microbiota during life-time is the first step in elucidating its role in health and disease. Bacteria belonging to the *Clostridium* sp. both commensal and pathogenic are highly abundant in the human gastrointestinal tract. However, during life the abundance of different predominant *Clostridium* species of healthy persons is not well studied.

**The aim** of the study was to assess the bacterial counts in *Clostridium* groups of intestinal microbiota of healthy humans within different life cycles.

#### Objectives

The study group comprised healthy infants (17male/8female; 11.9±0.6 month), children (13m/12f; 5.3±0.2 year-old), adults (4m/21f; 48.2±6.6 year-old) and elderly (9m/14f; 72.9±5.0 year-old).

#### Methods

DNA from fecal samples was isolated by using QIAamp DNA stool mini kit (Qiagen). The counts (plasmid copy number/g feces) were detected by real-time PCR with specific primers.

#### Conclusions

Four dominant *Clostridium* groups (*C.leptum*, *C.perfringens*, *C.coccoides* and *C.difficile*) were detected. Infants were more colonized with *C.perfringens* group bacteria in comparison to children, adults and elderly ( $p<0.001$ , for all respectively). Children were more colonised with *C.coccoides* group bacteria in comparison to elderly ( $p=0.005$ ). The counts of *C. leptum* group bacteria were highest in adults than in elderly and infants ( $p<0.001$ , both respectively). *C.difficile* prevailed in elderly and infants than in adults (10/23 (43.4%), 7/25 (28%) vs. 0/25 (0%),  $p<0.001$ ,  $p=0.005$ ; respectively).

Our study confirms that the quantitative composition of human intestinal *Clostridium* groups changes during life differentially. This data may be used for comparable analysis of the gut microbial composition between healthy persons and persons with particular disorders in different age groups.

**FEMS-2017**

**Microbial communities**

**MICROBIOLOGICAL COMMUNITIES OF THE WATER OF GELLÉRT BATH  
(BUDAPEST, HUNGARY) STUDIED BY CLASSICAL AND SPECIAL  
CULTIVATION TECHNIQUES**

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**Background**

Thermal baths are unique water environments combining a wide variety of natural and anthropogenic ecological factors. There is limited information on the microbiology of thermal baths in their complexity, tracking community shifts from the thermal wells to the pools.

**Objectives**

In the present study the “normal” microbial community of the well and the pool water in Gellért bath was studied in detail.

**Methods**

To isolate bacteria 10% R2A and minimal synthetic media (with „bath water”) with agar-agar and Gelrite were used after prolonged incubation time, moreover polyethylene blocks covered with media were also applied. Strains were identified by sequencing their 16S rRNA gene after grouping them by ARDRA.

**Conclusions**

On each applied media and from each sample the dominance of  $\alpha$ -Proteobacteria was characteristic though their diversity differed among samples. The largest groups in the pool water belonged to the *Tistrella* and *Chelatococcus* genera. The most dominant member in the well water was a new taxon, its similarity to *Hartmannibacter diasotrophicus* as closest relative was 93.62%. From each sample novel species could be isolated belonging to genera *Nocardioides*, *Rhizobium* and *Brevundimonas*. *Deinococcus* and low G+C Gram positives appeared only in the pool water. In order to cultivate novel taxa the best method was the combined use of minimal synthetic media, special cultivating techniques and increased incubation time.

**FEMS-1591**

**Microbial communities**

**CO-OCCURRENCE OF ATOPOBIUM VAGINAE AND GARDNERELLA VAGINALIS IN MALE URETHRA INDEPENDENTLY OF SEXUAL EXPOSURE**

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**Background**

Bacterial vaginosis (BV) is the most common genital infection among women of reproductive age. It is characterized by a transition in vaginal microflora from *Lactobacilli* to anaerobic bacteria.

**Objectives**

Though BV has been strongly linked to sexual behaviour, an endless controversy over the sexual transmission of BV remains elusive. Hence, we aimed to investigate prevalence and co-occurrence of BV-associated bacteria in urethras of healthy adult men in accordance with sexual exposure by using species-specific PCR.

**Methods**

First-catch urine specimens, representative of urethral swabs, from 114 healthy male volunteers were collected between January-June 2014, in Konya/Turkey. DNA was extracted using QIAamp mini kit (Qiagen, Germany) from pellets of 15 mL of urine. *Lactobacillus crispatus*, *Lactobacillus jensenii*, *Lactobacillus gasseri*, *Lactobacillus iners*, *Gardnerella vaginalis*, *Atopobium vaginae*, *Peptoniphilus* spp., BVAB1, BVAB2, BVAB3, *Megasphaera* type I, and *Prevotella* spp. were investigated by species-specific PCR assay.

**Conclusions**

Of 114 participants, 49 were sexually-unexperienced, and 65 were sexually-experienced, and all were circumcised. The most common species were *Peptoniphilus* spp. (53.5 %), *L. iners* (50.8 %), *G. vaginalis* (50.8 %), and *A. vaginae* (20.1 %). There was no significant relation between any BV-associated bacterium and sexual exposure. The only significant difference was in the co-occurrence of *A. vaginae* and *G. vaginalis* ( $p=0.025$ ) in the male urethra independently of sexual exposure. This result was compatible with similar reports studied on females. Since both *A. vaginae* and *G. vaginalis* are dedicated as key microorganisms in BV pathogenesis, more comprehensive studies on male counterparts are warranted.

## **FEMS-1901**

### **Microbial communities**

#### **VALIDATION OF THE INTELLICAP® SYSTEM AS A TOOL TO STUDY CHANGES IN THE SMALL INTESTINAL MICROBIOTA IN A DIETARY INTERVENTION STUDY**

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#### **Background**

The gut microbiota is involved in regulation of host metabolic and immune pathways, and is an important target for dietary interventions. Human microbiota studies are mainly based on the analysis of fecal samples. The microbiota in the small intestine differs substantially from the fecal microbial community, but is at least as relevant for human health. Nevertheless, this ecosystem has barely been studied due to the invasive sampling procedures. The IntelliCap® system is an electronic medical device for the site-specific delivery of drugs in the gastrointestinal tract. The IntelliCap® system has been adapted to aspirate liquid from its environment, and may thereby offer a minimally-invasive tool for sampling from the human small intestinal tract.

#### **Objectives**

To explore whether the IntelliCap® system can be used as a tool to study the human small intestinal microbiota composition.

#### **Methods**

A randomized cross-over controlled dietary intervention trial in 10 healthy male volunteers will be performed in January 2015. The study is approved by the Ethics Committee and subjects signed informed consent. Two diets are used: a three-day high-protein versus high-carbohydrate diet, each preceded by a washout diet. These diets aim to target microbiota with different fermentation requirements. The IntelliCap® capsule is administered after both intervention periods to collect a distal small-bowel sample. Fecal samples are collected from the same individuals. Microbiota composition is analyzed by sequencing using Illumina technology and dedicated bioinformatics.

## **Conclusions**

The IntelliCap® system may be applied to generate important new insights into the role of the human small intestinal microbiota in health and disease.



**FEMS-0477**

**Microbial communities**

## **SPECIES DISTRIBUTION OF NON TUBERCULOSIS MYCOBACTERIA ISOLATES FROM NORTH OF IRAN**

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### **Background**

Nontuberculous mycobacteria (NTM) differ from tuberculous mycobacteria as most of them are ubiquitous and saprophytic. A few are considered to have the potential to infect humans. The immunological status of a person determines the advance of disease. On the other hand among environmental factors, water plays the main roles as a resource of contamination in transferring this group of microorganisms to human.

### **Objectives**

The aim of this study is to identify and isolate NTM in the different region of Iran surface water.

### **Methods**

62 water samples were collected from different regions of Northern Iran. Cetyl pyridinium chloride (cpc) 0.01% was used to decontaminate the water samples. After enrichment using filtration, all samples were incubated on Lowenstein-Jensen (LJ) medium at temperatures of 30 and 37 °C. Classical culture, biochemical and enzymatic methods are described for the identification of mycobacteria. *hsp65* gene has more variability in its sequences, compared to the some more conserved genes in NTM, for identification of mycobacteria to species level.

### **Conclusions**

There were different *hsp65* gene PCR-RFLP profiles produced by digestion with *Bst*ElI and *Hae*III. Dominant isolates were *M. fortuitum*. Other isolates of *Mycobacteria* were differentiated using *hsp65* genes PCR-RFLP. This study indicates that notable percentage of water sources are contaminated with mycobacterium species. The exposure of immuno-compromised individuals to these sources can have crucial consequences. Interestingly the prevalence of mycobacterium species differs in diverse geographical areas.

**FEMS-2586**

**Microbial communities**

## **ENHANCEMENTS IN DNA-BASED DETECTION OF RARE SPECIES OF BACTERIAL COMMUNITIES BY PRETREATMENT USING GERMICIDAL ALKALINE CHEMICALS**

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### **Background**

In natural bacterial communities, the number of species existing at a low density levels is large and harbors the most of uncharted genetic information in nature. However, simple metagenomic sequencing methods can reveal those information for actively growing species unless a special method such as deep-sequencing or copy-number normalization is employed.

### **Objectives**

In this study, we aimed to develop a method increasing relative abundance of rare-species genomes while reducing that of predominant species. We hypothesized that treating community samples by a germicidal agent normalize copy number of genomes in metagenome samples.

### **Methods**

We treated soil and insect samples by alkalinizing chemicals including quicklime (CaO), NaOH, and KOH, which kills microbial cells in general. For each sample treated with different concentrations of alkalinizing chemicals, bacterial community was analyzed by t-RFLP and pyrosequencing of 16S rRNA gene, and by enumeration and identification of culturable bacteria.

### **Conclusions**

There were slight decrease of species richness in t-RFLP profile with stronger treatment (0-13% w/w CaO). However, overall diversity estimation (Shannon-Weaver diversity index) showed a steep increase at 3-5% CaO treatment. Chao1 estimates of richness for pyro-reads increased from 639±121 to 2,933±103 by optimal CaO treatment for soil samples. Rarefaction analysis on pyro-reads implied that richness of the untreated sample reached saturation while other richness values did not reach saturation. Shannon-Weaver diversity for pyro-reads increased from 1.8 to 6.7 by the optimal treatment. It could be concluded that the suggested treatments can increase

sensitivity of detecting rare species so that richness estimates increase more than five folds.

**FEMS-1182**

**Microbial persistence and chronic infections**

## **LONG-TERM SURVIVAL OF ENTEROCOCCUS FAECIUM OUTSIDE THE HUMAN HOST**

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### **Background**

*Enterococcus faecium* is a Gram-positive commensal of the gut of human and animals. However, in the last few decades it has acquired resistance to several antibiotics and has become an important cause of nosocomial infections. *E. faecium* can survive outside the human host for an extended period of time, which increases its ability to spread throughout the hospital and cause outbreaks.

### **Objectives**

Our goal is to uncover the mechanisms used by *E. faecium* to survive outside the human host.

### **Methods**

Using prolonged batch culture in brain heart infusion broth (BHI) or incubation in nutrient-free phosphate buffered saline (PBS) we determined the capacity of two clinical *E. faecium* strains (E745 and Aus0004) to survive at 20°C and 37°C.

### **Conclusions**

We show that temperature has a critical role in the long-term survival of *E. faecium*. At 37°C viable counts in PBS drop by 90% after 2 – 3 days while at 20°C the viable counts remain stable for 5 days. During prolonged culture in BHI, viable counts decrease by 90% after 7 days, but at 20°C viable counts remain stable for 10 days. Our finding suggest that *E. faecium* has specific mechanisms to survive outside the human host for a prolonged period of time. Currently, high-throughput functional genomic experiments are being performed to identify the mechanisms which contribute to survival of *E. faecium* in nutrient-poor conditions at ambient temperature.

**FEMS-1633**

**Microbial persistence and chronic infections**

## **DEVELOPMENT OF A SIMPLE ANIMAL MODEL TO STUDY CHRONIC PSEUDOMONAS AERUGINOSA INFECTIONS**

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### **Background**

Chronic infectious disease is increasingly recognized as a major public health threat. The development of novel, more effective antimicrobial therapies is of utmost importance. Antimicrobial drug discovery suffers from high attrition rates in later stages of drug development due to a lack of *in vivo* effectiveness of candidate drugs. Validation in an *in vivo* infection model early in the process is likely to remedy this problem. However, animal models that enable the *in vivo* study of chronic bacterial infections are typically complex and generally involve higher animals such as mice.

### **Objectives**

We sought to develop a simple animal model that would allow the study of a prolonged bacterial infection by a clinically relevant pathogen.

### **Methods**

We selected the opportunistic pathogen *Pseudomonas aeruginosa* and the crustacean host *Daphnia magna*. *D. magna* juveniles were infected with varying doses of a virulent strain of *P. aeruginosa*. While the highest infectious doses resulted in acute toxicity and complete mortality within 2 days after exposure, lower doses did not cause increased mortality compared to uninfected control populations up to 2 weeks after exposure. Infection with a *P. aeruginosa* strain expressing green and red fluorescent proteins allowed visualization of infection sites using fluorescence microscopy. Labeled bacteria were still readily identified 2 weeks after exposure, revealing a stable chronic infection.

### **Conclusions**

Together, our results strongly suggest that the simple *P. aeruginosa*-*D. magna* model is a suitable tool for studying chronic bacterial infections *in vivo* and support its further development for use in validation of candidate antimicrobials.



**FEMS-0654**

**Microbial persistence and chronic infections**

## **NEW INSIGHTS INTO PERSISTERS METABOLISM**

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### **Background**

Persistence is defined by a phenotypic switch occurring at low frequency ( $\approx 10^{-6}$ ) and generating a subpopulation tolerant to antibiotics. After removal of the antibiotic, persistent bacteria are able to regrow. Persistence is therefore thought to be responsible for chronic infections and could play a key role in the survival of biofilms to antimicrobials. Despite these observations, molecular mechanisms underlying persistence remain unclear.

### **Objectives**

The aim of this study is to shed light on the molecular mechanisms by which phenotypic variability is generated in bacteria, allowing them notably to switch from an antibiotic susceptible state to a persistent state.

### **Methods**

In a first approach, the main experimental parameters affecting *E. coli* persistence in our experimental conditions (aeration rate, overnight culture duration, growth medium, carbon source, ...) were identified. A standard and reproducible method for measuring persistence was then set up and validated. Using this method, we are currently investigating persisters genetics and metabolism. Approaches combining the use of deletion mutants, fluorescent transcriptional reporters and biosensors are used to determine which metabolic pathways are activated/shut down in persisters.

### **Conclusions**

Our data confirmed that persisters consist of highly heterogeneous populations. In addition, different classes of antibiotics appear to generate different persistent populations, characterized by the activation of distinct metabolic pathways and stress responses. This indicates that molecular mechanisms underlying persistence are not unique and a variety of mechanisms are involved. Population (FACS analyses) and

single-cell (time-lapse microscopy) behaviours are currently analysed to get further insights into persisters mechanisms diversity.



**FEMS-2147**

**Microbial persistence and chronic infections**

**DURING CHRONIC INFECTIONS SALMONELLA ACQUIRES MUTATIONS IN REGULATORS OF METABOLISM AND VIRULENCE GENE EXPRESSION**

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**Background**

*Salmonella enterica* is responsible for diverse infections in mammals, including chronic infections. For *Salmonella enterica* serovar Typhi, the causative agent of typhoid fever, up to 6% of patients, will become life-long carriers.

**Objectives**

Despite the importance of chronic infections, the molecular mechanisms of chronic host association are poorly understood. The objectives of this study are to elucidate the mechanisms employed by *Salmonella* for establishment of chronic infections.

**Methods**

Mice were infected with low doses of *Salmonella*, and the development of chronic infections was followed over the course of the infection. After six weeks of infections mice were sacrificed and the livers and spleens were analyzed for the presence of *Salmonella*. Dominant clones of livers, spleens and fecal samples were whole genome sequenced to identify adaptive mutations.

**Conclusions**

Most clones had acquired one or two non-synonymous point mutations (single nucleotide polymorphism, SNP). Most SNP's were associated with metabolism and virulence regulation. For example, a dominant clone from a fecal sample had a SNP in *kdgR*, a repressor of genes of the Entner-Doudoroff pathway, indicating metabolic adaptation in this clone. Re-infection experiments revealed that the *kdgR*-SNP mutant exhibited rapid and superior colonization of the mouse intestines resulting in a super-shedder phenotype within 1-2 weeks post-infection. Furthermore, the KdgR-SNP regulator protein could not be induced leading to constitutive repression of its target genes, supporting metabolic remodeling in the *kdgR*-SNP mutant. In conclusion, during a short chronic infection, *Salmonella* adapts to its host by modifying the activity of central regulators of metabolism.

**FEMS-2176**

**Microbial persistence and chronic infections**

## **FEEDBACK BETWEEN MICROBIAL INTERACTIONS AND EVOLUTION IN CHRONIC CF INFECTIONS**

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### **Background**

Although many infections are assumed to be monoclonal it is becoming clear that interactions between bacterial pathogens and other microbes at the infection site may alter pathogen behaviour. We have limited understanding of the evolution of microbial interactions and the extent to which interactions constrain or promote evolution of the interacting partners during the course of infection.

### **Objectives**

Chronic airway infections in cystic fibrosis (CF) patients provide opportunities to study microbial evolution and its relation to microbial interactions. The CF airways are nearly always infected with a number of microorganisms, including *Pseudomonas aeruginosa*. CF infections are associated with genetic adaptation of *P. aeruginosa*, and to understand the adaptive mechanisms, we have focused on two transmissible *P. aeruginosa* lineages – DK1 and DK2 - that share a similar natural history: They both entered the Copenhagen CF Clinic more than 40 years ago, adapted in one or a few patients and subsequently spread to a large number of patients. Our aim is to uncover the genetic changes that determine the apparent dominance of these clones, and to investigate in which way microbial interactions shape the evolutionary pathways.

### **Methods**

The systematic collection of bacterial isolates from lung expectorates of CF patients at the Copenhagen CF Clinic since 1973 has made it possible to investigate within-host evolution of both lineages in relation to both inter- and intraspecies interactions.

### **Conclusions**

We show that the microbial CF community context impact evolution of the individual lineages, and that evolved changes within individual species can modify the behavior of the infection community

**FEMS-2043**

**Microbial persistence and chronic infections**

**THE ROLE OF CHLAMYDOPHILA PNEUMONIAE IN THE ETIOPATHOGENESIS OF SCHIZOPHRENIA: RELATIONSHIP BETWEEN SCHIZOPHRENIA BRAIN-DERIVED NEUROTROPHIC FACTOR (BDNF) AND NEUROTROPHIN-3 (NT-3) A WORLDWIDE RETROSPECTIVE STUDY**

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**Background**

It's known that, in the occurrence of schizophrenia, genetic predisposition, neurodevelopmental disorders and environmental factors play role. It was suggested that synthesis of brain-derived neurotrophic factor (BDNF) and neurotrophin3 (NT3) were upregulated from monocyte/macrophages infected with Chlamydomphila pneumoniae, considered among environmental factors.

**Objectives**

In this study we aimed to show at the worldwide level, for the first time, the relation of C.pneumoniae infection, BDNF and NT3 levels .

**Methods**

In this crosssectional retrospective study 50 patients with shizophrenia and 35 healthy controls(HC) were included. The C.pneumoniae DNA was investigated by RT-PCR from PBMC and IgA, IgG, IgM were investigated by immunofluoresans in both

group's serum. BDNF and NT3 levels were determined by ELISA. Chi square student's t and Mann Whitney U tests were used for statistical analyses .

### **Conclusions**

A significant difference was found between PG and HC cases for chronic C. pneumoniae infection seropositivity ( $p < 0.05$ ). The mean serum BDNF and NT-3 levels were found to be significantly lower for PG members than for HC members ( $p < 0.001$ ). We couldn't detect any C. pneumoniae DNA in PBMC of 85 participants. In conclusion, although in HG, compared to HC, the presence of chronic C. pneumoniae infection was found remarkable high, BDNF and NT3 levels, were found significantly low in PG compared to HC, which is the first international study in the basis of C. pneumoniae, schizophrenia relation, and the detection of BDNF and NT3 levels, a conviction wasn't reached and we suggest that new studies, especially based on cohort and performed with large series including these three parameters supported by molecular methods are needed.

**FEMS-0928**

**Microbial persistence and chronic infections**

**MOLECULAR MECHANISM ANALYSIS OF PERSISTERS FORMATION WITH A CELL DIVISION MARKER**

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**Background**

Persisters are multidrug tolerant cells due to their suppression of cell division. When bacterial population is exposed to antibiotics, persisters could survive and cause for the recurrence of infections. Although molecular mechanisms of bacterial persistence to antibiotics have been studied, a crucial gene had not been identified. Since the mechanisms of persisters formation are redundant, analysis targeting a specific single gene is not appropriate for persister researches.

**Objectives**

The objective of our research is to develop a marker for persisters that enable to isolate persisters and to analyze an exhaustive gene expression.

**Methods**

To distinguish non-dividing cells from dividing cells, fluorescent protein YFP and CFP were fused at the both N and C terminals of FtsZ as a cell division marker. The fusion proteins emit CFP fluorescence in non-dividing cells, moreover they emit fluorescence resonance energy transfer (FRET) signal at Z-rings in dividing cells. This mechanism enables to visualize non-dividing cell and dividing cell. Non-dividing persisters were isolated using a cell sorter and their transcriptome was explored using a microarray.

**Conclusions**

The fusion FtsZ emitted FRET fluorescence at the Z-ring. Sorted cells in low FRET signal fraction exhibited about 50 times higher survival rate after exposure to antibiotics (ofloxacin) than cells in high FRET signal fraction. Hence, our method attained an objective. Transcriptome analysis suggested following three hypotheses; i) persisters take up energy by anaerobic respiration, ii) membrane protein is sensing changes of the environment, iii) clinical thread is not caused only by persistence, but also by secretion system.

**FEMS-1647**

**Microbial persistence and chronic infections**

**IN VITRO EVOLUTION OF PERSISTENCE BY CYCLIC TREATMENT WITH AMINOGLYCOSIDES IN PATHOGENIC ESCHERICHIA COLI AND SALMONELLA**

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**Background**

Bacteria can evade antibiotic eradication by forming transiently antibiotic-tolerant phenotypic variants, called persisters. Persistence is linked to the recurrent and chronic nature of many bacterial infections, but mechanisms underlying this phenomenon have mainly been investigated in non-pathogenic *Escherichia coli* lab strains.

**Objectives**

Infections by many Gram-negative pathogens are becoming increasingly difficult to treat due to resistance towards multiple antibiotics. Since aminoglycosides often retain activity against these strains, there is revived interest in their clinical use. We investigated the evolution of persister levels in uropathogenic *E. coli* and *Salmonella* under periodic aminoglycoside treatment.

**Methods**

Previously, a method for the experimental evolution of persistence by cyclic antibiotic treatment was optimized in our lab. Here, we apply this method to investigate the evolution of persister levels in uropathogenic *E. coli* UT189 and *S. enterica* Typhimurium SL1344.

**Conclusions**

Once daily, high dose aminoglycoside application rapidly selects for mutants with extremely high persister levels, while resistance levels (minimum inhibitory concentrations) remain unchanged. Likewise, mutants show increased persister levels in biofilms. Moreover, evolved uropathogenic *E. coli* mutants display cross-tolerance to fluoroquinolones, although high persister levels of *S. enterica* Typhimurium mutants seem antibiotic-specific. The dosing scheme of our *in vitro* experiments is roughly comparable to current clinical practice, underscoring the relevance of our findings. Future clinical evaluations of antibiotic dosing regimens

should include an assessment of persistence, as this could be an overlooked factor in failure of antibiotic therapy.

**FEMS-2651**

**Microbial persistence and chronic infections**

## **A SURVEY OF NASAL CARRIAGE RATE OF STAPHYLOCOCCUS AUREUS IN DIALYSIS PATIENTS**

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### **Background**

Staphylococcus aureus is the most common cause of cutaneous pyogenic infection. Some groups of individuals seem to be particularly prone to colonization with S.aureus. For example, patients undergoing chronic hemodialysis and users of illicit intravenous drugs

### **Objectives**

The purpose of this study was determination of nasal carriage rate of S.aureus in dialysis patients

### **Methods**

This survey was carried out in a descriptive analytic cross-sectional research in 2012. 71 patients of chronic renal failure were selected who had undergoing dialysis in dialysis ward of kashan. After obtaining consent form ,Specimens with swabs were performed from anterior nares nose of the patients .the questioners having demographic and related risk factors were filled through interviewing.anitimicrobial resistance S aureus of positive cases were determined by disk diffusion

### **Conclusions**

The patients were 41 females (53.2%) and 36 males (46.8%). The mean of age in patients was 48.43 years and the mean of duration of dialysis was about 23.54 months. 12 cases were nasal carriers of S.aureus (16.9%). There was no significant statistical correlation between sex,age, job, under lying disease and duration of dialysis and nasal carriage. The most resistance of S.aureus was seen to meticillin and the most sensitivity was to rifampin. Resitance to cefazolin ,cotrimoxazole 33.3,vancomycin 50% and cloxacillin 75%.there was no S aureus was fully sensitive to vancomycin.

Considering to 16.9% frequency of S aureus carriage among dialysis patients and 50% resistance to vancomycin ,periodic examination and proper treatment due to antibiogram results is recommended.



**FEMS-2595**

**Microbial persistence and chronic infections**

**DOES TONSILS AND ADENOID TISSUE PLAY A RESERVOIR ROLE FOR COLONIZATION OR INFECTION WITH HAEMOPHILUS INFLUENZAE IN PATIENTS WITH CHRONIC ADENOIDITIS OR CHRONIC TONSILITIS?**

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**Background**

*Haemophilus influenzae* is a commensal bacteria but can cause severe infections including meningitis, sepsis, bacteraemic pneumoniae, otitis media, sinusitis, and chronic bronchitis

**Objectives**

The present study was conducted to investigate the reservoir role of tonsils and adenoid tissue for colonization with *H.influenzae* bacteria in patients with chronic tonsillitis and adenoiditis and genotyping analysis of the strains isolated from both sites of the same patient

**Methods**

A total of 200 specimens (100 tonsils and 100 adenoid tissue) were taken from 100 chronic tonsillitis and adenoiditis patients who undergoing adenotonsilectomy. All samples were bacteriologically cultured and Pulsed field Gel electrophoresis (PFGE) method was applied for all *H.influenzae* isolates which revealed a high growth in tonsil and adenoid tissue.

**Conclusions**

**Results.** It was found that 82 out of 100 (82%) tonsils and 79 out of 100 (79%) adenoid tissue cultures a total of 161 samples were exhibited 274 bacteria from different bacterial species. *H.influenzae* strains were formed % 32 (87/274) of the total bacterial growth. A twenty eight isolates from each of tonsils and adenoid tissue of 28 patients (56 isolates) were analyzed by PFGE method which revealed that 52 out of 56 (92.85%) *H.influenzae* strains of 26 patients were similar whereas four strains of two patients were different.

**Conclusion.** The tonsils and adenoid tissue are playing a reservoir role not only for colonization with *H.influenzae* and other different bacterial species but each site is also a reservoir for others and vice versa. For our opinion *H.influenzae* strain could be a key for prevention of chronic tonsillitis and adenoiditis.



**FEMS-2956**

**Microbial persistence and chronic infections**

**MYCOBACTERIAL PERSISTENCE: USING MICROFLUIDICS AND SINGLE-CELL MICROSCOPY TO INVESTIGATE HOW MYCOBACTERIA SURVIVE ANTIBIOTIC EXPOSURE**

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**Background**

Up to a third of the world's population is estimated to be infected with *Mycobacterium tuberculosis*, the causative agent of tuberculosis (TB). A significant ability of this pathogen is its capacity to adapt to and survive stress. This may in part be the reason why TB treatment requires such a lengthy time – at least 6 months of antibiotics. Both of these observations may result from a propensity for mycobacteria to form antibiotic tolerant persisters.

**Objectives**

We investigate how growth rate affects mycobacterial survival when exposed to antibiotics, with an initial assumption that growth arrest is a major mechanism promoting bacterial survival under these conditions.

**Methods**

A microfluidic platform was used that allows observation of single-cells of *M. smegmatis* by widefield microscopy, with constant flow of defined medium, and rapid switching between different conditions. The drug rifampicin was used to dissect persister formation due to its bactericidal properties, as well as its clinical relevance in TB treatment. Nutrient limitation was used to determine how this affects persister formation.

**Conclusions**

Growth rate of individual cells prior to rifampicin exposure appears to have no impact on their ultimate survival, however growth arrest is clearly observed during antibiotic exposure in all cells. Nitrogen limitation and starvation appear to significantly decrease survival, whilst carbon limitation appears to have little effect. This work, though preliminary, may enhance our understanding of how persisters form in mycobacteria, and help in the design of novel intervention strategies to combat tuberculosis.



**FEMS-1228**

**Microbial persistence and chronic infections**

**FREQUENCY OF ANTIBIOTIC APPLICATION DICTATES RAPID EVOLUTIONARY ADAPTATION OF BACTERIAL PERSISTENCE**

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**Background**

Microorganisms employ different strategies to cope with changing environments. Typically, a dedicated sensor perceives a specific environmental trigger and signals an adaptive response. However, if changes are rare and/or abrupt, maintenance and time lag of sensory systems become too costly. Consequently, bacteria evolved the capacity of switching stochastically between phenotypes to hedge their bets. Such a bet-hedging strategy based on a non-genetic switch between a sensitive growth state and an antibiotic-tolerant, non-growing persister stage, protects bacteria against eradication by antibiotics. Persistence causes antibiotic therapy failure and is therefore responsible for the chronic and sometimes incurable nature of infections.

**Objectives**

Little is known about the evolutionary forces that determine persister levels. We therefore aim to delineate the evolutionary dynamics of persistence. Furthermore, we want to explore the theoretically suggested and intuitively appealing correlation between the level of persistence and the frequency of antibiotic application.

**Methods**

We used experimental evolution in *Escherichia coli* to demonstrate rapid, reproducible and reversible changes in tolerance levels that optimize the population's fitness in function of the treatment frequency. Daily antibiotic attacks select for single-nucleotide changes that confer high persister levels (up to 100 %) and shorten persister lag phase. Persister levels decrease again in the absence of selective pressure. When time between antibiotic strikes increases, intermediate persister levels evolve that correlate with treatment frequency.

**Conclusions**

We show that persistence is an evolvable phenotype that quickly adapts to environmental dynamics, which may have important implications for developing optimal treatment strategies of chronic bacterial infections.

**FEMS-1539**

**Microbial persistence and chronic infections**

**GENOMIC AND TRANSCRIPTOMIC CHARACTERIZATION OF BURKHOLDERIA PSEUDOMALLEI WITHIN-HOST EVOLUTION IN FIVE CHRONICALLY INFECTED AUSTRALIAN CYSTIC FIBROSIS PATIENTS**

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**Background**

Cystic Fibrosis (CF) is a genetic disorder that results in impaired mucociliary clearance of inhaled microorganisms. Over time, the airways of CF patients become colonised with different microorganisms, resulting in infections that cannot be eradicated. An uncommon pathogen of CF patients, but of rising significance, is *Burkholderia pseudomallei* (Bp). Bp is an opportunistic environmental pathogen that causes melioidosis, a potentially fatal disease that is endemic in most tropical regions, particularly northern Australia and Southeast Asia. Melioidosis is being increasingly described in CF patients returning from or living in melioidosis-endemic regions. Presentations of melioidosis in CF patients range from acute infection with rapid deterioration, to a chronic infection with slowly progressive deterioration in lung function.

**Objectives**

Unfortunately, the molecular basis for this chronic presentation remains entirely unknown.

**Methods**

To address this knowledge gap, we performed whole-genome sequencing on paired longitudinal Bp isolates from five Australian CF patients. To complement the genetic analysis, we have also performed stranded, deep sequencing of the transcriptome using Illumina RNA-seq. To best mimic the bacterial expression profiles in the CF lung, isolates were grown in an artificial sputum medium and RNA was extracted at late-log phase.

**Conclusions**

We will be reporting on the genomic and transcriptomic changes detected in the CF isolates, which have been shaped by within-host evolution. Similarly to common CF pathogens such as *P. aeruginosa*, we have observed a rise in multi-antibiotic resistance and changes to virulence factor expressions and metabolism.

**FEMS-2034**

**Microbial persistence and chronic infections**

**ANTIBIOTICS STRONGLY AFFECT COST AND BENEFIT OF COMPETENCE IN BACILLUS SUBTILIS**

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**Background**

*Bacillus subtilis* differentiates stochastically into the state of competence for transformation. Transformation is thought to speed up adaptive evolution in the presence of antibiotic stress.

**Objectives**

Here we address the question if the state of competence confers a strong benefit during episodic antibiotic treatment and acts as a persister state.

**Methods**

Using head-to-head competition experiments between strains with different rates of differentiation into the state of competence, we found that the cost of competence increases with the probability of differentiation. Competence was highly beneficial when competitors were treated episodically with antibiotics that inhibit cell wall synthesis, translation, transcription, or proton motive force. This benefit was independent of transformation. Time-kill kinetics in conjunction with single cell time lapse microscopy showed that the state of competence is a persister state. Addition of antibiotics did not increase the fraction of competent cells, indicating that competent *B. subtilis* are type II persisters. Finally, we tested whether the generation of phenotypic heterogeneity in terms of competence served as a bet-hedging strategy when *B. subtilis* was treated episodically with antibiotics. In competition experiments between strains with different probability of differentiating into the state of competence we found that populations that generate phenotypic heterogeneity minimize the cost of competence under benign conditions while increasing the probability of survival when challenged with antibiotics.

**Conclusions**

We conclude that *B. subtilis* uses a bet-hedging strategy for increasing its chance of survival when challenged with antibiotics by stochastically differentiating into the state of competence which acts as a persister state.



**FEMS-1557**

**Microbial persistence and chronic infections**

**QUANTITATIVE ANALYSES OF PHENOTYPIC STABILITY OF STAPHYLOCOCCUS AUREUS SMALL COLONY VARIANTS ISOLATED (SCV) FROM CYSTIC FIBROSES (CF) PATIENTS**

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**Background**

Formation of small colony variants (SCV) of *Staphylococcus aureus* is a strategy (not only for *Staphylococcus aureus*) to overcome antibiotic treatment and to escape the immune system. This phenotype can persist for long time periods in patients with low pathogenic activity. One problematic feature of SCV is the ability to switch easily back to a more virulent phenotype.

**Objectives**

The aim of the study was to establish a test to quantify the ability of SCV's to switch back to a non SCV phenotype and to test several clinic isolates.

**Methods**

Five *Staphylococcus aureus* SCVs, isolated from five different patients with cystic fibrosis were tested. All SCV's were not able to grow in Mueller Hinton (MH)-broth. From each isolate 500 single colonies grown on Lysogeny Broth (LB)-Agar for 24 h showing SCV colony type were inoculated in 200µL MH broth for 24h. Growth in MH was confirmed by plating on MH agar plates and identification of *S. aureus* via MALDI-TOF analysis.

**Conclusions**

Four of the five SCV isolates showed stable phenotype with only 0-0.8% phenotype changes. In contrast one SCV changed the phenotype in 25.2%, which means at least one bacterial cell restored the ability to grow on/in MH media.

This test method is based only on one SCV characteristic and does not document if there is a total reoccurrence of the original wild type phenotype. Nevertheless it is an easy assay, and it will be a possibility to investigate the influence of different conditions on the stability of SCV's.



**FEMS-1884**

**Microbiology education**

## **SCIENTIFIC EXHIBITIONS: THE MICROBIOLOGY FROM THE LAB TO THE STREET**

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### **Background**

In November, the University of the Basque Country (UPV/EHU) organized the 14th edition of the Science Week (ZientziaAstea). This annual event, aimed at audiences of all ages, tries to bring scientific concepts, methodologies and innovations to a non-specialist public by means of children's workshops, scientific shows, conferences and other activities. The microbiology has been present in this event from the first edition.

### **Objectives**

In ZientziaAstea, we aim to bring the public diverse aspects of microbiology, to clarify certain misconceptions and to show its importance in our daily lives.

### **Methods**

Every year, our Department's staff prepares materials and activities and attends the questions of school children and general public in a stand. Initially, the proposals consisted mainly in microbial cultures, photographs, diagrams and microscopic preparations. Year-to-year, proposals have become more complex, including models, videos, and posters with a common thread (hydrothermal vents, food, interactions) or hot topics (ebola). In the latest edition, we have incorporated the workshop *Microorganisms working* to refute the bad image of microorganisms and to highlight their benefits and their ecological and industrial importance.

### **Conclusions**

We have observed a good response for assistance and acceptance by the public in these activities, but we have also detected the need to renew the issues and approach. As consequence, for the 15th edition we are preparing the workshop *Small microbiology for small scientists* that, through games and activities, will try to introduce children the microbial world (the microbial diversity in a big drop of water or in our body, the bacterial growth, etc...).

**FEMS-2625**

**Microbiology education**

**WORKING ON ACCESSIBILITY OF MICROBIOLOGY EDUCATION FOR DEAF COMMUNITY: DEVELOPMENT AND USE OF A NEW SCIENTIFIC GLOSSARY IN SIGN LANGUAGE**

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**Background**

University should be an open and accessible environment to all people, where knowledge and opinion can be exchanged. Deaf community often finds scientific language as a hard barrier blocking their education. Knowing that most of Sign Languages are usually extremely colloquial, the development of an international scientific Sign language seems to be as useful as necessary.

**Objectives**

This work aims to develop a handy and rigorous Sign Language that allows to all Deaf community establish an independent university education and research avoiding the problems associated with continued use of interpreters.

**Methods**

We have created a sum of 320 signs for terms commonly used in science and related with Microbiology. These terms have been attributed to a sign, maintaining a logic in the use of Sign Language and a scientific adequacy. All the signs have been validated and used both by qualified interpreters and by deaf students from Complutense University of Madrid. All the terms have been included in a glossary and diffused firstly by using the Complutense University "Virtual Campus" and then on the web page of Spanish Society for Microbiology.

**Conclusions**

The main objective of this project has allowed us to develop teaching resources adapted to deaf students. The glossary has been applied to design dynamic accessible slides including explanations in Sign Language and illustrative multimedia tools making easier the educational process to deaf students in both, theoretical and practical lessons on Microbiology.

**FEMS-2933**  
**Microbiology education**

**ANTIBIOTIC RESISTANCE: DEFINING THE SCIENTIST'S RESPONSIBILITY.**

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**Background**

Antibiotics transformed global health and the fields of scientific discovery. The use of antibiotic tools is ubiquitous but the rise of antibiotic resistance is threatening modern medicine. Scientists are asking manufacturers to stop selling antibiotics as growth promoters; new initiatives to stimulate antibiotic research; and a better educated public and clinicians to champion responsible use of antibiotics.

**Objectives**



The next generation of biologists and microbiologists are taught the mechanisms of antibiotic resistance in formal academic settings. They learn about the 'urgent need' for new antibiotics, from a teaching and scientific research community urging responsible use of antibiotics. Yet students attend laboratory-based practical classes relying heavily on antibiotics. Should the scientific community examine how learning opportunities encompassing antibiotics and antibiotic resistance are incorporated across university curricula to ensure we provide young scientists with consistent and synoptic information? Should future scientists be encouraged to use antibiotics more responsibly and sparingly in both educational and research settings?

## **Methods**

The roundtable discussion will debate whether we give conflicting messages to young scientists and will examine how learning opportunities encompassing antibiotics and antibiotic resistance questions are provided. Should we begin to practice what we preach and develop alternative practical classes where antibiotics and antibiotic resistance are not treated as disposable teaching tools?

## **Conclusions**

Self scrutiny of our own educational practices will give us a stronger voice when we ask society to use antibiotics more responsibly and may encourage development of new technologies offering similar benefits and functionality as current tool set that is based on antibiotic resistance.

## **FEMS-2534**

### **Microbiology education**

#### **ACTIVITIES OF THE EDUCATION & COMMUNICATION DIVISION OF THE SPANISH SOCIETY FOR MICROBIOLOGY (D+D SEM): FOCUS ON YOUTH**

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#### **Background**

The Spanish Society for Microbiology (*Sociedad Española de Microbiología*, SEM; [www.semicrobiologia.org](http://www.semicrobiologia.org)) has 11 specialised Divisions. Among them, the most recently created and transversal is the Education and Communication Division (*Docencia y Difusión*, D+D SEM; [www.semicrobiologia.org/ddm](http://www.semicrobiologia.org/ddm)). It hosts 180 members committed to Education in Microbiology at several educational levels within Spain and to promote our Science to Spanish-speaking people worldwide via the internet and social networks.

#### **Objectives**

D+D SEM aims to cover different degrees of formation:

#### **Methods**

First, a work team establishes links with professionals at primary and secondary schools, offering assessment and support to Science teachers about microbiological subjects, and aiming to the content of official teaching modules and text books. D+D SEM has recently published a book of educative short stories about Microbiology aimed to children.

Second, D+D SEM promotes the elaboration and open exchange of materials and resources for Microbiology Education for University degrees, including videos, podcasts, games, blogs, interactive on-line tools, webpages and innovative approaches for Microbiology teaching. Remarkably, our Division organizes yearly workshops on “Initiation to Research in Microbiology”, in which 20 brilliant and motivated University students selected nationwide share with senior Microbiologists their passion for research.

Third, at the postgraduate level, young researchers can find support and information on Master degrees, training courses or job opportunities at the Group of Young Researchers ([sites.google.com/site/jovenesinvestigadoressem](http://sites.google.com/site/jovenesinvestigadoressem)), hosted by D+D SEM.

#### **Conclusions**

We seek to share experience and coordinate our effort with similar initiatives in other countries.





**FEMS-1833**  
**Microbiology education**

**WORKSHOPS ORGANISED BY STUDENTS FOR STUDENTS: AN ORIGINAL  
MULTIDISCIPLINARY APPROACH FOR TEACHING MICROBIOLOGY AND  
INFECTIOUS DISEASES.**

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**Background**

During the last decades, efforts have been made for the development of innovative teaching in medical studies.

**Objectives**

To describe an Innovative Teaching Unit (ITU) implemented for the students being in the 4<sup>th</sup> year of medical studies in Angers University, France.

**Methods**

This ITU includes 20 original topics. Each year, each topic is attributed to 4 groups containing 4 students. After choosing an issue in the field of their topic, they have to organise and conduct a 3-hour workshop, to present a poster or an oral communication in a local congress, and to produce a written report. Each topic is supervised by two teachers from different medical specialties to ensure a multidisciplinary approach. The topic called 'Major sanitary crises' (MSC) was included in the ITU 4 years ago.

**Conclusions**

Two or three meetings between 2 teachers and students are necessary to prepare the workshops. In 8 of the 12 sessions of MSC that have been already completed, the students chose a topic in the field of microbiology and infectious risk (bioterrorism, Ebola virus, risks of waterborne diseases after a hurricane, and the use of antibiotics in intensive livestock farming). Students used original supports to animate workshops (homemade videos, street interviews, live staging, interventions of professionals and quiz)

This ITU, combining problem-based learning and project-based learning, allows a total implication of students in the organization of workshop and obtaining original

teaching and supports on subjects that are not namely taught during their curriculum and prepare them early to scientific communications

**FEMS-1841**  
**Microbiology education**

**PLACE OF MICROBIOLOGY IN AN INTERDISCIPLINARY TRAINING  
PROGRAMME (MASTER LEVEL) ABOUT THE MANAGEMENT OF GLOBAL  
HEALTH AND NUTRITIONAL SAFETY**

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**Background**

"Manimal" is a pioneering interdisciplinary training programme (ITP) (Master level) based on the "One World, One Health" concept promoted by international organizations (WHO, OIE, FAO), with an interdisciplinary approach for the management of global health and nutritional safety.

**Objectives**

Microbiology is a topic of great importance for global health and food safety and quality. Our objective was to determine the place of microbiology in this ITP.

**Methods**

This international ITP is organised in 5 thematic teaching units (TU): epidemiology, health: organisation, management and communication, biological and chemical risks, production chains, nutritional contamination and transfers. We particularly identified the interactions developed between microbiology teaching and other TUs in an interdisciplinary perspective.

**Conclusions**

Traditional lectures (face teaching or e-learning sessions) about microbiology are included in the "biological and chemical risks" TU. However, microbiology is also involved in interdisciplinary trainings via student groups around case scenarios (problem-based learning) and visits of production sites to understand the control measures implemented against the development of *Listeria monocytogenes*

(interactions with the production chains TU). Two case scenarios are proposed: "Listeriosis and dairy industry" (interactions with the nutritional contamination TU) and "Antimicrobial resistance" (interactions with all other TUs). Groups containing 4 or 5 students with various initial educations (doctors, pharmacists, veterinarians, biologists, and agricultural engineers) and from different geographic origins (Asia, Africa, Europe) are constituted with the aim to make them solve the problems together. We envisage now to introduce microbiology in tutored projects (project-based learning) and to diversify the microbiological topics in the case scenarios.

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**Microbiology education**

**THE SMALL WORLD INITIATIVE AT MCGILL: ENGAGING UNDERGRADUATES IN SCIENTIFIC DISCOVERY AND CROWDSOURCING THE SEARCH FOR NEW ANTIBIOTICS**

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**Background**

The Small World Initiative (SWI), spearheaded by the Yale Center for Scientific Teaching, is an international, multi-institutional initiative which brings authentic scientific research addressing the real-world problem of antibiotic resistance to undergraduate life science students. SWI students culture soil microbes, screen for antibiotic activity against safe relatives of ESKAPE pathogens, and characterize their positive isolates by classical methods and 16s rRNA gene sequencing.

**Objectives**

McGill launched the SWI program in a sophomore Microbiology laboratory course of 105 students in fall 2014.

**Methods**

During the fall 2014 semester, the students collected soil samples from urban and suburban Montreal and cultured 2,500 bacterial isolates. Following activity screening, 104 isolates were prioritized for further characterization using classical biochemical and microscopy methods and molecular characterization.

**Conclusions**

Of the 104 isolates selected for further characterization, 94 gave positive results in 16S PCR, and of these, 71 yielded high quality 16S sequences. The isolates were equally divided between Gram-positive and Gram-negative, with a wide variety of genera represented. The students were highly engaged throughout the process and reported a high level of enthusiasm in course evaluations.

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**Microbiology education**

**THE DEVELOPMENT OF 'VIRUSES: A PRACTICAL RESOURCE FOR POST-16 BIOLOGY TEACHERS'**

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**Background**

Practical science activity in the classroom is an essential part of science education in secondary schools. Microbiology, the study of microorganisms (including bacteria, fungi, algae and viruses) relies heavily on practical activity in its teaching. Viruses have significant impact on human activity, encompassing human disease and epidemiology, molecular biology research, treatment of disease and phage-related damage to bacterial starter cultures in food production. These phenomena relate to many aspects of the school teaching specification.

**Objectives**

To develop a new resource containing background information on viruses including real world case studies, three practical activities about viral infection and molecular techniques for diagnosis of viral infection, and guides for teachers, technicians and students.

**Methods**

The booklet underwent extensive formative evaluation in preparation for launch, including trials with students and teachers as well as considerable development in a laboratory setting, in and out of the classroom environment.

**Conclusions**

Subsequently, 'Viruses: a practical resource for post-16 biology teachers' was launched (2013) as an 80-page colour educational resource aimed at post-16 biology education. The resource is freely available to Society for General Microbiology members and is about to undertake extensive summative evaluation.

